

nature

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Some seasonal greetings



To our leader writer:

May all your issues be central ones; may every corrective measure be at once too little and too much.

To book blurb writers:

May all your wares continue to be of interest to highly qualified specialists, students and the layman alike.

To those eminences who require their secretary to type after their name MA, PhD, ScD, FRIC, FInstP, FICE, CEng, FRSEdin:

May you excuse the modesty of our response.

To Hills Road, Cambridge:

May your access to our columns be as easy as everyone else in the world assumes it is.

To all our London subscribers:

May we never again send you *Nursing Times* instead of *Nature*. Think of what the poor nurses had to wade through.

To all directors of big government laboratories:

May you never again need to be thanked for granting permission to your staff to publish.

To all those whose notepaper is headed:

US Department of Health, Education and Welfare,
Federal Ventures Office,
Bureau of Diseases,
Common Cold Eradication Program,
Viral Infection Project,
University of South Chicago,
Western Campus,
Emily Smoot Building,
Department of Biochemistry,
Sub-department of Sneeze Research,
Room 3581

May you get a box number.

To all authors of rejected papers:

Even though you are 'naturally disappointed' by our decision, may you maintain your custom of 'not being in the habit of writing to complain'.

To all those who telephone us and find us unavailable:

May you ring us again; we won't ring you.

To all our readers:

May your grumbles find their way to us; we may not be on the same grapevine as you.

To a scientific jet-setter:

May you look on the messages board at Heathrow airport. We don't send letters to you at your department any more.

To the editors of new journals:

May there always be interdisciplinary subjects crying out for a new journal dedicated to rapid dissemination of results; may you find a goodly number of Nobel Laureates for your editorial board.

To our referees:

May we never again send an expert on tadpoles a paper on general relativity. And if we do may it never take a month for him to let us know.

To Tom Jukes and Kenneth Mellanby:

May you find issues for your columns as black and white as we make your faces.

To our printers:

May we come to some agreement on the spelling of millennium.

To Professor J. L. Jinks:

May we never again call you Professor J. L. Links.

To our sub-editors:

May you remember the difference between partly and partially, and between compared with and compared to; may you put b.p. but bc.

To readers of News and Views:

May you find your own work described therein—and not in slighting tones either.

To our authors:

May you put your conclusion in your first paragraph. After that can come the introduction. The middle bit then goes at the end.





The Flowers Report: opportunities missed

Dr R. H. Mole, Director of the MRC Radiobiology Unit at Harwell, offers some personal reflections on the recent nuclear power report of the Royal Commission on Environmental Pollution

THE Report's main conclusion is that there are substantial environmental objections to a nuclear power programme on the scale envisaged (paragraph 534) and that we should not rely for energy supply on a process that produces such a hazardous substance as plutonium unless there is no reasonable alternative (paragraph 535). In an earlier paragraph (186), the conclusion is much more categorical: although aware of the advantages that nuclear power offers the Commission did not think that these outweigh the risks described. Routine electricity production by nuclear means, however, appears to be regarded as unobjectionable.

It is necessary to consider important omissions from the Report, so important as to appear to warrant asking whether these conclusions are justified.

The human cost

The routine environmental effects of nuclear power may well be much less damaging than those of fossil fuelled power stations... The discharges of radioactivity from nuclear stations, both to air and water, are radiographically insignificant and there are no discharges of offensive gases or particulates. (191). ... the aspects of nuclear development which cause most dissension tend to be those that relate not to normal operation but to the possibility of abnormal events such as accidents at nuclear installations or malevolent acts against them. (10).

Although the Report says that the Commission "should be concerned not so much with the individual risk as with the total effect of possible accidents" (173) this total effect is never considered. The human cost of nuclear power is considered and enumerated only in relation to single examples of what were said to be very unlikely events, an exceptional release of radioactivity into the sea at Windscale (139) or into the atmosphere from a serious reactor accident (263-268). There is nothing in the Report to show that the Commission considered or enumerated the total casualty rate from reactor malfunctions and small accidents which will occur much more frequently than exceptional severe accidents, or even from the routine operation of power reactors without malfunction. Is it a mistake to conclude that, when the Commission concentrated on exceptional occurrences, it did not know whether it had addressed itself to the real problem of nuclear safety or only to a minor aspect of it?

In relation to the possibility of terrorist activities the Commission con-

tented itself with stressing that purloining enough plutonium to make a crude bomb is bound to happen sooner or later but did not attempt to deduce the likely or possible consequences in terms of numbers of casualties. Thus on this question the Royal Commission with all its expertise and resources has added nothing to what has been said repeatedly over the past few years by the ordinary journalist.

Energy Strategy

Chapter IX on Energy Strategy considers energy needs and alternative means of satisfying those needs but nowhere considers the human costs of getting energy by any means, except to take for granted that nuclear power is basically hazardous. Much of the Report's anxiety seems to be based on "the assumed necessity of securing steadily increasing energy supplies" (197) as illustrated in Table 14. But no facts are provided to enable the reader to consider what methods of supplying energy might be the safest to the population and the least damaging to the environment if there was no need to increase energy supplies at all. Chapter VI is said to relate nuclear and non-nuclear hazards (16) but it does not do this for alternative means of getting energy.

The Commission's failure to compare the human costs of alternative sources of energy and to consider the total effect of possible accidents can make its anxieties about exceptional serious accidents seem wholly unbalanced. This can be illustrated as follows. The current death rate from accidents amongst employees of the National Coal Board is in round terms 50-60 per year. The number of cancer deaths from a particular example of failure of containment of Caesium-137 at Windscale is given as perhaps fifty in total (spread out over many years). Such an event is said not to be at all likely and its consequences are used to illustrate the need for the strictest containment of radioactive wastes for very long periods (139), as if the number of casualties was something out of the ordinary and perhaps unacceptable to the Commission.

The large radioactivity release considered as an exceptional but very serious event is of 10^7 Ci Iodine-131, 10^6 Ci Caesium-137 and so on, and would give 110-350 deaths from cancer (263-268). This is the same as the number of accidental deaths in coal production in UK over a 2-6 year period

at the current annual rate. The probability of release of 10^7 Ci Iodine-131 is 10^{-7} per reactor year (the Report's Figure 15). One hundred reactors each of 1,000 MW would produce twice as much electricity as the whole of that generated by all means in UK in 1975 (462, 468), and the probability of release of 10^7 Ci Iodine-131 would then be once every 100,000 years. On this count alone nuclear power would appear to be more than 10,000 times safer than coal power.

Such a comparison is obviously inadequate since it is between a most exceptional event and a routine annual occurrence. It shows very clearly how misleading it may be to concentrate on exceptional events with large consequences and to ignore much commoner events each with relatively small consequences. Differences in emphasis on these contrasting sources of risk were in fact noted by the Royal Commission as the reason for inconsistencies in evidence on the policy for siting reactors (296), but the Commission seems to have failed to appreciate the significance of the matter for itself.

Consistency in assessing the acceptability of risk

The Royal Commission emphasised the need for a body with statutory responsibility for endorsing the basic standards for radiological protection on the national behalf (223). Such a body must be clearly independent of any interests promoting developments involving radioactivity, it is agreed, but the conclusion can be questioned that "in view of the importance of the issues it should be a body constituted specifically for radiological protection" (223). Whatever the history of how the basic standards were chosen, they are expressed in terms of tissue dose and, therefore, can be specifically related to risk.

Technically this relation must be as exact as current scientific understanding will allow, but "the importance of the issues" at the present time is not so much the degree of technical accuracy as the acceptability of the corresponding risk. This cannot be wholly determined by any body specifically constituted only for radiological protection, as that phrase would normally be understood, because acceptability is not a radiological matter. The non-technical essence of the choice of basic standard is to set the corresponding level of risk at that point on the scale of risk which makes it acceptable in the light of all other sources of everyday risk in work and generally.

The Royal Commission recognised that the collective action of involved government departments was inad-

equate for providing advice on energy strategy (515) and recommended the need for an independent high-level advisory body on the subject (535). All the more reason for recognising the need for a central body to consider and report publicly on the level of acceptability of risk in relation to the various means by which the great variety of national needs could be satisfied, energy supply being but one of these. Consistency in assessing acceptability of risk is required not only within the energy field but also between the energy field and other major national activities not excluding the practice of medicine.

For example, at the present time roughly as much Iodine-131 is administered to patients, and from them is released into the environment each year, as would be released on average per year over the 100,000-year period within which one most exceptional release of 10^7 -Ci Iodine-131 might be expected from a hundred nuclear reactors routinely generating electricity.

Public reaction to risks

A major factor in the Royal Commission's approach, although not made wholly explicit, is expressed clearly enough in the Report: risks of events that affect people individually or in small numbers, it says, "are fairly readily accepted as inevitable", but events "that cause death or injury to many people at one time . . . have, in proportion to the numbers affected, a much greater impact in creating public concern" (172 and see 270).

The Commission, in emphasising so strongly the exceptional event and accepting almost without question the safety of routine discharges of radioactivity into the environment, seems to have adopted the same attitude. Even if the members of the Royal Commission regarded themselves as in some sense representatives of the public or trustees of the national interest, there seems no reason why they should accept these public attitudes as given and unchangeable and appropriate for themselves. It was surely within their remit to attempt to change public attitudes by explanation and reason. This would certainly have to be a function of a central body to consider acceptability of risk, as suggested above, if it was to succeed in providing consistent guidelines on emotive subjects.

Responsibilities and experts

The Royal Commission found it difficult to obtain independent but expert advice "since the acknowledged experts are often themselves involved in the related developments" (163). So far as assessing the effect of pollutants, including radioactivity, "we can do no better than require that the agencies concerned are expert, open and in-

dependent, and such as to ensure that necessary research is undertaken and that assessments are appropriately revised in the light of new knowledge. The requirement reflects on organisational arrangements" which are required (188).

The International Commission on Radiological Protection (ICRP) "is only as good as its members, and it is vitally important that these should continue to be appointed independently of the approval of their national governments and purely on the basis of their professional standing amongst their scientific peers . . . We hope that there will continue to be a strong British representation on the ICRP . . ." (203).

A main problem, however, with the Commission's recommendations for systematising responsibilities is that they will still further reduce the possibilities of finding informed and at the same time independent experts, that is independent of interested organisations.

Bodies with statutory responsibilities as proposed by the Commission, such as the strengthened NRPB, the Nuclear Waste Management Committee, the Nuclear Waste Disposal Corporation, the strengthened Health and Safety Executive (531) and HMPI (527), will clearly attract to themselves the best experts they can find. Any British members of ICRP will inevitably be found to belong to NRPB as, even now, three out of four current British members of ICRP do. Each such body will get financial support from government sources. Are they in the least likely to criticise each other publicly as the Royal Commission said they should be free to do? If an organisation has statutory responsibilities it must have a "party line" to which its employees conform. Even if, like NRPB at present, it is a national centre for advice without statutory responsibilities, it must ensure consistency in the advice it gives. Is this conducive to criticism from within of scientific or technological bases of action?

What the Royal Commission quite rightly seemed to desire was to maximise the possibilities for what might be called informed assent and dissent. This requires not only publicly available information but the existence of individuals who are not beholden to organisations with statutory responsibilities and who are technically educated and intellectually free to pursue argument and the advance of knowledge without the confinement of terms of reference or administrative boundaries.

Is there a big enough reservoir of such individuals for national needs? It wouldn't seem so for radiobiologists if major reports in Britain, like the Royal Commission's, and in the USA, like the

Rasmussen Report on Reactor Safety, were each radiobiologically defective, and the same reasoning can be applied to any other scientific field covered by the Royal Commission Report. The only way of remedying this sort of deficiency is to see that universities and polytechnics pay sufficient attention to the field of knowledge and that research councils play their part.

Research priorities

The Royal Commission regarded the arrangements for initiation and co-ordination of research as of the greatest importance but in no case was the priority of research discussed in relation to the quantitative importance of the subject matter as it relates to risk. Research could well be justified if a more than negligible risk is to be assessed but, if routine nuclear power is already known to be unobjectionable as suggested by the Commission, would research on its hazards be needed? It could hardly be justified merely by legislative requirements for annual surveys of discharges of radioactivity (251).

Debate and accessibility of fact

In its last few paragraphs the Royal Commission Report quite rightly stressed the importance of public understanding of the long term issues of unusual range and difficulty raised by nuclear development (521), and the need for open and deliberate weighing of risks and costs of embarking on a major nuclear programme against those of not doing so (522). But such an open political process will generate only hot air and useless emotion unless there is an agreed basis of technological fact from which to start. The scientific convention is to provide a reference for every possibly arguable fact, and this systematic buttressing by checkable publications is something the dyed-in-the-wool scientific reader rather sadly misses. The enquirer cannot make the checks he may desire: for example, the source of only ten of 25 Figures and three of 14 Tables is acknowledged, and even then a specific reference is provided for only four of the 13 sources acknowledged. Perhaps a Royal Commission Report, especially if it deals with complex issues, is not a suitable place for a comprehensive reference list, but it must be a retreat from established scientific methodology if a Report of a Commission on highly technical questions is without adequate references because then its authority is simply the eminence of its individual members. This is a special problem in the present case, because many readers of different backgrounds have recognised serious defects in the Report.

For instance, the Royal Commission

was concerned that insufficient research and attention had been given to atmospheric pollution by radioactivity (234), yet there is no evidence that the Commission knew of the more than 100 scientific papers on this subject published from the Environmental Sciences and Medical Division of AERE.

Conclusions

The Report of the Royal Commission on Environmental Pollution fails to provide (i) any evaluation of the relative level of risk of nuclear power from accidents and from normal operations, (ii) any corresponding evaluation of

risk from other means of energy supply, (iii) any recommendation for a means of considering the level of risk in relation to acceptability on the national behalf.

Without (i) it would seem that the Royal Commission cannot know whether or not, in its emphasis on exceptional occurrences, it has tackled the real problem. Without (ii) its examination of Energy Strategy is woefully inadequate. Without (iii) its recommendation for a strongly based national body to consider radiological protection leaves that body with no standards by which to take decisions.

The Report concluded that plutonium must not be produced on a large scale until it is demonstrated that the plutonium can be taken care of safely. This is bound to be generally agreed. But safety is an open-ended concept and it must be a matter of judgment to decide whether what is known does or does not amount to the demonstration of a sufficient degree of safety. No criteria are provided by which such a judgment could be made.

Without all these, how can there be a deliberate weighing of the risks, costs and advantages of a major nuclear programme? □

Flowers on Flowers

Sir Brian Flowers, who recently stepped down as Chairman of the Royal Commission after 3½ years, made his first public comments on reaction to the nuclear power report when he spoke at the British Nuclear Energy Society last week. Some extracts:

On the disposal of radioactive waste: "I have occasionally been asked what we meant by demonstrating a method beyond reasonable doubt. There appears to be agreement that a full programme of research might take a decade or more, but we argue that in any case we have that time available before becoming irretrievably committed to nuclear power as a major component of our electricity supply. I would suggest that what is needed in addition to a vigorous, systematic research programme is to choose and to set aside as soon as possible at least one specific site for which there is a consensus of geological, hydrological and other relevant opinion that it is acceptable for inaccessible disposal. It need not be the one eventually adopted, but it would provide a fall-back position. This, too, is a problem that exists irrespective of the future of nuclear power."

On alternative energy strategies: "The crucial long-term issues are on the one hand the competition between nuclear energy and coal, and on the other hand between both and renewable resources; and also between high electricity and therefore high heat-waste, on the one hand, and vigorous conservation measures on the other. Whether it is looked at from the industrial or the environmental point of view there is a very strong element of nuclear versus the rest. In these circumstances it is vital that responsible advice to Ministers should be seen to be independent of the major factions in the argument. I believe that it should be a matter of public

concern that this is not at present the case."

On the possible effects of illicit activities: "On this issue, alone amongst all those we studied, we were given very little help by official bodies . . .

" . . . Probably the Government already has all the legal powers it would need under the Anti-Terrorist Acts of 1974 and 1976. Is Mr Eadie [Minister of State at the Department of Energy] saying that the Government has consciously resolved that the use of these powers is acceptable to safeguard the future electrical power industry? And that the changes then implicit for our society will be amply offset by the economic benefits of nuclear power? Perhaps he is, and perhaps he is right; but how are we to know unless we can discuss the matter? The Royal Commission does not know the answers to these questions. It merely considers them important and asks that they be discussed before we develop a reliance on nuclear power. If the Government continues to skulk behind the Official Secrets Act we may not know until it is too late to wish that it were otherwise."

On nuclear weapons proliferation: " . . . Thanks to the vested interests of the nuclear weapons states; thanks to the huge R&D resources already devoted to the development of nuclear power; thanks to the almost universal belief in a continuing abundance of cheap electricity, only recently shown to be a myth by the oil sheiks; thanks to the ensuing lack of attention paid to the need for energy conservation, the exploration of more meaningful long-term energy strategies, and the development of alternative sources; and on the global scale, thanks above all to the lack of any effective population control—it is simply not possible in 1976 to state with the required

degree of certainty that the world need not be dependent on nuclear power beyond the end of this century. A severe lack of energy, and the continued division of the world into rich and poor which some would then envisage, might itself be extremely dangerous, even to the extent of bringing in the very nuclear hazards we were seeking to understand and if possible to lessen."

On the question of developing a commercial fast breeder reactor: "Judging by experience from other prototype reactors we shall still be learning a great deal from it in five or even ten years' time. In any case, it is now unrealistic to suppose that the first full-scale breeder reactor, the so-called CFR-1, could be started in under three or four years, even given the approval tomorrow. That gives ample time for the nuclear debate to come to a conclusion. I believe one should contemplate building any such reactor on a remote site contiguous with its own recycling plant so that there would be no need, after the first loading, for substantial quantities of plutonium to enter or leave the site. It is hardly likely that the result would justify the adjective 'commercial', but I believe it could be done in an environmentally acceptable fashion provided there is no commitment to an ongoing programme."

"But I see no need for undue speed, even if one believes there is no avoiding fast reactors and the plutonium economy they entail. Thermal reactor development has proved to be within the capacity of the United Kingdom, but only just. Fast reactor development is likely to be beyond our resources. It seems to me essential that if we pursue it at all we should do so on a European basis. I can see no reason for wishing it were otherwise now that we are fully committed to the European Community . . ."

Nature's Christmas issue a year ago carried an article entitled 'Naming the Loch Ness monster'. This year, **Carl Sagan** writes on 'Detection times and number densities of rare mobile organisms: application to Loch Ness'.

If there are any, could there be many?

IN simple collision physics, when one moving object may collide with a number of stationary and dissimilar targets, the mean free time for collision is $t = (n\sigma v)^{-1}$, where n is the number density of stationary targets, σ is the mean collision cross-section and v is the relative velocity (assumed constant). If targets are also moving, a factor of order unity multiplies the denominator of this equation; for example, if all objects have Maxwell-Boltzmann velocity distributions, the multiplier is $2^{1/2}$.

This same equation, slightly modified, can be used to deduce the number density in three dimensions of a distributed rare mobile organism from the mean free time between sightings of this organism by a stationary or moving observer. In this case the collision cross-section $\sigma = \pi r^2$ where r is the target visibility range—the linear distance over which the target is within the resolving power of the observer; or the distance to optical depth unity in the enveloping medium; or the distance to the horizon, whichever is least. When t is measured by a stationary observer, the mean distance between organisms will then be

$$S = 2(3t r^2 v/4)^{1/3} \quad (1)$$

If the total volume in which the organisms are contained is V , the total population of organisms in this volume is

$$N = V/(\pi r^2 v t) \quad (2)$$

These relations assume that the organisms being observed are neither attracted to nor repelled by the observer, and that the observer has chosen a typical locale in the organism's habitat—for example, not in the vicinity of concentrations of predators or prey. Under these circumstances equations (1) and (2) provide expectation values for the mean separations and total numbers of organisms. In the common case that the geometry is two- rather than three-dimensional (as, for example, for land animals and to a significant extent even for birds), n is replaced by A' , the column density of organisms, σ is replaced by r , $S = 2(rvt/\pi)^{1/2}$ and $N = A/rvt$, where A is the area of the total habitat.

As a practical application, consider the interesting and controversial set of observations suggesting the presence of large organisms in Loch Ness¹. Sonar and underwater stroboscopic photography in 1975 imply $t \approx 10^4$ to 10^5 s

for some unidentified large animal of characteristic dimensions 10 m. We adopt $t \approx 3 \times 10^4$ s, but bear in mind the impression of the observers that in 1975 the organisms may have been attracted by the observational equipment and therefore that the appropriate t is significantly longer. Because of the turbidity of the loch, $r \approx 10$ m; and a rough estimate of the swimming velocity of the unknown animals is $v \approx 3 \text{ m s}^{-1}$.

Equation (1) then immediately gives $S \approx 0.4 \text{ km}$, a very large mean separation distance. The total volume of Loch Ness is approximately 10^{16} cm^3 , whereupon, from equation (2), $N \approx 300$. Because of the cube root in equation (1), our estimate of S is reasonably independent of the uncertainty in t . But our uncertainty in estimating the total population is proportional to the uncertainty in our estimate of t . If the targets were indeed attracted to the observing apparatus, then N is less, and a conservative estimate places N between several tens and several hundreds. Curiously, this is just the estimate derived independently from biomass calculations, assuming that the diet of the unknown organisms is exclusively migratory salmon² or exclusively non-migratory prey³. While the agreement of these two quite different sets of calculations—from the waiting time for observation in 1975 and from the biomass of the loch—should not be overstressed, the agreement does tend to support the contention that there is a real population $\approx 10^{2 \pm 1}$ of large organisms

inhabiting Loch Ness.

The negative photographic results for 1976 have been attributed⁴ to the sharply diminished fish population and significant increase in temperature in the loch, connected with the 1976 drought. If the 1976 results were not anomalous, we might deduce $t \geq 10^7$ s, and $N \leq 1$. However, sonar encounters at larger r were recorded in 1976.

The nature of these organisms seems still more uncertain than their existence, but it appears more likely that they are a minor variant of a fairly abundant contemporary taxon than, for example, the only surviving group of aquatic Mesozoic reptiles. The large calculated separation distances in a medium as turbid as Loch Ness suggests that the organisms might be equipped with echo locator organ systems and may communicate at audio frequencies. Hydrophones should be an important adjunct to any continuing study.

Similar calculations of organism spacing and loading density could be made on other planets, were macro-organisms to be discovered there—as, for example, on Mars with the Viking lander imaging system. □

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² Scott, P., and Rines, R. H., *Nature*, 258, 456 (1975).

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⁴ Mackal, R. P., *The Monsters of Loch Ness* (The Swallow Press, Chicago, Illinois, 1976).

⁵ Sheldon, R. W., and Kerr, S. R., *Limnol. Oceanogr.*, 17, 796 (1972).

⁶ Scheider, W., and Wallis, P., *Ibid.*, 18, 343 (1973).
⁷ Wyckoff, C. W., Private communication (1976).



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USA

New career for Glomar Explorer?

The National Science Foundation is looking into the possibility of converting the Central Intelligence Agency's Glomar Explorer into a deep sea drilling vessel. Colin Norman reports from Washington.

WHEN it became known in March last year that the Central Intelligence Agency (CIA) had used a vessel called the Glomar Explorer to scoop bits of a sunken Soviet submarine from the bottom of the Pacific, a number of scientists in the United States expressed concern that the affair could jeopardise flourishing Soviet-American cooperation in deep sea research. A particular worry was that the Soviet Union might sever its ties with the deep sea drilling project (DSDP), a highly successful ocean drilling programme which the National Science Foundation was then endeavouring to turn into an international research enterprise. Ironically, however, the DSDP may turn out to be the chief beneficiary of the CIA's salvage operations.

Last week, the National Science Foundation (NSF) announced that it is studying the possibility of converting the Glomar Explorer into a drilling vessel for possible use in the 1980s. NSF officials, and their clients in many earth science laboratories, are hoping that the Glomar Explorer will provide a cut-price way of carrying out drilling projects which are beyond the capabilities of the present DSDP vessel, the Glomar Challenger. Though it is far from certain that the NSF will be able to get the funds to turn the vessel into a research ship, it is the only use for the ship which is now under serious investigation.

The Glomar Explorer was constructed for the CIA by Global Marine Inc (the same company which built and operates the Glomar Challenger, hence some of the worry about Soviet scientific reaction), for a sum reported to be about \$300 million. Though publicly billed as a special seabed mining ship built by Howard Hughes, the reclusive billionaire, it was designed for the specific purpose of salvaging a Soviet submarine which sank in about 1,700 feet of water 750 miles north-west of Hawaii in 1968.

The ship recovered bits of the submarine in June 1974—how much is still a matter of some dispute—and when reports of the operation began leaking to the press nine months later, the CIA suddenly found itself with an almost new vessel for which it could

find no further use.

In the meantime, earth scientists have begun to think about what should follow the present phase of the DSDP. The choice boils down to either dispensing with deep sea drilling entirely or mounting a programme of drilling in presently inaccessible areas such as potential oil-bearing regions, the ocean trenches and under the Arctic sea, a programme which would require a new vessel with greater capabilities than the Glomar Challenger. It would require considerable lifting capacity to handle the long drill strings needed for drilling in ocean trenches, and a thick hull to penetrate the Arctic ice. Moreover, since drilling in the ocean margins entails the risk of striking gas or oil deposits, the vessel would have to be able to handle complex and heavy apparatus to prevent possible blowouts. Construction of such a vessel would probably cost between \$60 and \$70 million, a price tag which probably puts it out of the reach of NSF's strained budget.

But Peter Wilkniss, the NSF project director for the DSDP, noted in an interview last week that "when you look at the requirements, it becomes obvious that the Glomar Explorer is almost ideally suited." It is 618 feet long, compared with 400 feet for the Glomar Challenger, and it is capable of lifting about 2,000 tons—more than 10 times the capacity of the Challenger. Moreover, its more powerful engines and greater stability would probably allow it to work in rougher seas.

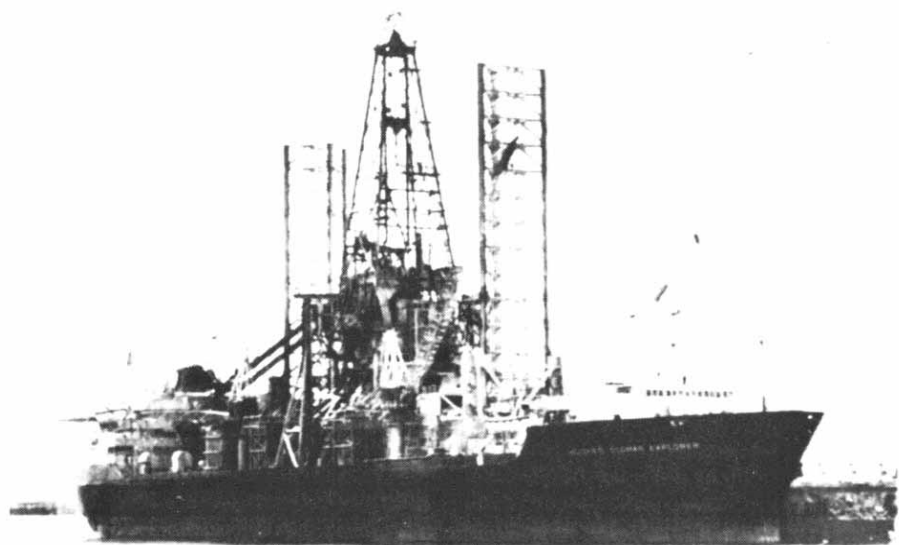
It is by no means certain, however, that the vessel could be converted

easily and cheaply from a salvage ship into a deep sea drilling vessel, and thus NSF has authorised Global Marine to conduct a preliminary study, under a \$75,000 contract, of the feasibility of converting and operating the Glomar Explorer. The results of that study should be available early next year. In the meantime, the Scripps Institution of Oceanography, which manages the DSDP under a contract from NSF, is looking at the scientific requirements of an expanded drilling programme.

So far, NSF has not given much thought to arrangements for operating the ship if it does decide to acquire and convert it. But, in view of the potentially high costs of operating the vessel—Wilkniss estimates about \$30,000–40,000 per day, twice that of the Glomar Challenger—there would be obvious advantages in seeking international participation.

In that case, the Glomar Explorer's past associations could pose a problem. Though the Soviet Union did not pull out of the DSDP after the submarine salvage attempt was made known, the operation is said to be a sensitive matter and some ocean researchers privately wonder whether the Soviet Union would participate in a venture for which the Glomar Explorer was a central feature.

It should also be noted that if NSF does decide to follow the present DSDP with a programme of drilling in the ocean trenches and on the continental margins, much of the work would be carried out within the 200-mile economic zones likely to be established as part of a Law of the Sea agreement. That would subject the research to a possible veto by coastal states in whose waters the Glomar Explorer may want to work. □



Glomar Explorer

USA

● The Joint Committee on Atomic Energy, the Congressional body which has reigned supreme over nuclear policy in the United States for nearly three decades, last week suffered a severe, possible fatal public battering. Already weakened by the loss of several key members through retirements and election defeats, and facing concerted attack from senators and congressmen seeking its abolition, the joint committee last week became the target of some well-aimed barbs hurled by Common Cause, an influential lobbying group.

In a lengthy review of the joint committee's activities, Common Cause says it has uncovered numerous examples of "procedural abuse", pro-nuclear bias, and failure to examine important issues. The harsh indictment may turn out to be an important factor in persuading Congress that the time is ripe to carve up the joint committee's responsibilities and divide them among other congressional committees, a move which is already being planned and which would have important implications for nuclear policy.

The joint committee derives much of its immense power from its unique status as the only Congressional committee which handles legislation in both the House and the Senate. Every bill related to nuclear power, no matter where it originates, must be referred to the joint committee, a fact which gives its 18 members a monopoly over nuclear legislation.

Formed shortly after the second world war to oversee the United States's nuclear weapons programme, the joint committee rapidly became one of the most powerful committees in Congress. For more than two decades its authority went largely unchallenged and legislation it approved was virtually guaranteed easy passage through the House and Senate. But, as nuclear power has turned into a controversial issue, the joint committee has attracted increasing fire from nuclear critics. It has been persistently accused of pro-nuclear bias, and in the past couple of years a few other Congressional committees have even begun to invade its turf by holding hearings on nuclear matters. (The joint committee is still the only committee with authority to consider nuclear legislation, however.)

Criticisms of the committee have recently given way to more strident calls for its complete abolition, and the Common Cause blast is no exception. The organisation's report pre-

sents evidence that the joint committee has given short shrift to the concerns of nuclear critics, failed to explore important nuclear issues which raise doubts about nuclear power, refused to take the lead in examining major policy questions—such as nuclear proliferation—and often used its unique Congressional position to rush legislation through both the House and Senate before



relevant studies have been completed.

Common Cause also notes that the committee has persistently voted for increases in nuclear spending, adding more than \$850 million to the budget of the Energy Research and Development Administration (ERDA) in the past three years alone. Those actions, Common Cause claims, can be explained in part by the fact that the committee is "dominated by representatives who have a vested interest in encouraging increased nuclear spending". In the last session of Congress, for example, 12 of the committee's members represented six states which together carried off more than half the funds distributed by ERDA.

The chief recommendation from Common Cause is that the joint committee should be scrapped and its responsibilities transferred to House and Senate committees which have broad authority over all energy research and development matters. The report is likely to give considerable impetus to moves already afoot which would accomplish precisely that objective.

Next month, when Democrats in the House of Representatives meet to consider their political agenda, Representative Jonathan Bingham will propose that the joint committee be scrapped and its responsibilities be consigned to other committees, probably the Science and Technology Committee or the Interior Committee. And a committee reorganisation plan

proposed recently for the Senate would transfer the joint committee's responsibilities for nuclear research and development to an expanded Interior committee, and its authority over nuclear regulation to a new Public Works and Environment committee. The reorganisation plan is expected to be debated by the Senate in January, as one of its first items of business.

● If the Joint Committee on Atomic Energy is scrapped by the new Congress, the nuclear industry in the United States would lose one of its most important political allies just as a number of matters deemed crucial to the industry's long-term interests are coming to a head. Among those issues is whether the United States should press ahead with the liquid metal fast breeder reactor programme, at present the highest priority and most lavishly funded energy research and development effort supported by the federal government.

In that regard, a report published last week by the General Accounting Office (GAO), an investigative agency of the Congress, is unlikely to help the LMFBR's political acceptability. The report estimates that it would cost some \$150,000 million to build the 128 breeder reactors which ERDA is now planning, compared with \$95 million to build coal plants of an equivalent capacity and \$128 million for light water reactors. That level of capital expenditure would be difficult for the electricity industry to raise without federal assistance.

● There has recently been a spirited debate in many countries about how to dispose of commercial nuclear wastes and keep them isolated from the environment for thousands of years. The disposal of nuclear wastes from military programmes in the United States is, however, an even bigger problem. Last week, the Energy Research and Development Administration announced that it hopes to start drilling next year in some 13 states to find suitable sites for commercial repositories. Though no sites have yet been short-listed, ERDA officials said that they hope to have the first repository in operation by 1985, and some six in operation in the 1990s. The total cost of the programme, which will handle some 330,000 cubic feet of material by the year 2000, is reckoned to be \$2,000 million. Under questioning, however, ERDA officials said that the total amount of waste generated by military programmes by 2000 will be about 11 million cubic feet, and the cost of getting rid of it could be as much as \$20,000 million.

Colin Norman

FRANCE

La Soufrière's first victim

Fierce disagreement characterised the debate amongst French scientists about La Soufrière, the volcano in Guadeloupe in the French Antilles. A report from La Recherche:

WHEN La Soufrière started to grumble in July 1975 the memory of the 30,000 victims who perished in the cloud of burning ash thrown out by Pelée Mountain in Martinique in 1902 made the question of evacuation particularly important. Some 72,000 people were living at the foot of La Soufrière, which is one of a group of volcanoes in the Caribbean island arc.

Its activity has been characterised by its andesitic nature, the relative viscosity of its magma, the long periods of calm between eruptions and particularly by the explosive nature that its eruptions can have. Although known eruptions (1797, 1836 and 1956) had produced only a slight rain of volcanic dust and released some steam, the authorities eventually did evacuate the local population as a preventative measure. But after a period of intense activity in July and August, the volcano seemed to quieten down again. Things should then have returned to normal; in fact the trouble had only just begun.

On October 27, the board of the Teaching and Research Unit (UER) of the Institute of Physics of the Globe (IPG) met to discuss with the Director of the Institute, M Allegre, the case of Haroun Tazieff, the famous French volcanologist. Allegre informed the board that he had decided to dismiss Tazieff (63) from his post as director of vulcanology at the IPG. Tazieff, he explained, had refused to guarantee that he would fulfil the commitments of the post, after being asked to do so several times and in particular at the beginning and end of August. That followed criticisms of Tazieff for going off to hunt for three Britons lost in the Andean Cordillera when 72,000 French people could have been in danger.

Although no vote was taken at the meeting those present unanimously approved the measure. As the IPG does not come under the administrative jurisdiction of the National Centre for Scientific Research (CNRS), Tazieff continued as a research director at CNRS; his team was anyway in the process of moving to Gif sur Yvette outside Paris to be on CNRS premises. The break with the IPG was complete, though, because Tazieff had no desire to remain any longer on its staff.

The episode marked the culmination of an unfortunate conflict between French scientists which had for several months taken precedence over the real problems confronting the people of Guadeloupe, already faced with considerable political, social and economic difficulties. The UER has given its official reasons for the dismissal of Tazieff, but some believe it may be the result of a quarrel which has been exacerbated by a series of petty disagreements over the differing interpretations of the phenomena observed on the volcano itself.

Tazieff's own somewhat strong words, which the press willingly echoed, started the quarrel and made it all the more bitter as time went on. In *Le Monde* (September 5) he declared that he "could expose the faulty reasoning which has led certain scientists not competent in vulcanology to put forward mistaken prognostications, which the volcano itself has proved wrong in the past six weeks, but I will only do so in a scientific journal." Five days later, there were even stronger words in the weekly *L'Aurore*: "Those who blocked my way are incompetent," he declared. "They are all perfectly competent in their own fields, but they should not try to interfere in vulcanology. If you have trouble with your liver you do not consult an ophthalmologist."

The confrontation deepened on the scientific plane. From July 1975 La Soufrière manifested an increasing number of the phenomena which have now died down again: a seismic shock in March, the appearance of new fractures, underground phreatic eruptions (in July), exceptional seismicity, stronger and more frequent eruptions (in August). Over this period, the number of seismic shocks registered rose from 30 in July 1975 to 209 in November, 607 in March this year, 1,220 in July and, in August, almost 6,000 tremors.

The scientific community agreed on these facts, but there was no unity about the diagnosis. On the one side, Professor Robert Brousse declared that he could no longer rule out the possibility of an explosive phase which could come very suddenly. John Tomblin of the University of Trinidad supported this viewpoint. On the other hand, Tazieff was more reassuring, saying that there was no need to fear a rapid development, and that the only risks for September were from the underground eruptions which endangered only the immediate vicinity



La Soufrière

of the volcano. "Any major eruption," he added, "will be preceded by warning signals for about a week."

On August 15, after a strong tremor of magnitude 4.6 shook Guadeloupe, the political authorities organised a massive evacuation of the population. Even though Allegre wished to hold a middle view between the two extremes, in effect he confirmed the alarmist views of Brousse when he sacked Tazieff, describing his statements as unscientific.

A committee of international experts met in Paris in the middle of last month, brought together by the CNRS to give its opinion on the surveillance programme for the volcano, its organisation, and the results obtained. During these meetings, members have been listening to the many French and international scientists who have been involved with La Soufrière.

It appears from the discussions that there is no danger in the short term of any sudden explosive phenomenon, and that all changes in the behaviour of the volcano should be easily registered by the modern instruments available. It is essential, the committee has noted, that the equipment at La Soufrière should be improved and reinforced. This means that the number of seismograph stations in the existing array should be increased from seven to about twenty, and that they should be set out around the circumference of the volcano. It was also agreed that magnetometers, tiltmeters and inclinometers should be installed.

The committee also advised that to utilise the installations best, a volcanologist and a petrologist should, as an experiment, be employed permanently in the area. They would look after the equipment, make daily observations and do regular analyses of the material thrown up by the volcano. This would avoid, the committee hoped, the danger of over-reacting when there were insufficient data. □

USSR

Bottling up dissent

Vera Rich reports on recent developments concerning dissidents in the USSR and Eastern Europe.

LEONID PLYUSHCH, a former prisoner of conscience who was interned in the Dnepropetrovsk psychiatric penal institution, visited London recently. His presence formed a rallying point for all those concerned in the cause of human rights and freedom of thought in the USSR and the Comecon bloc.

Plyushch, a cybernetician who was arrested in 1972 for "anti-Soviet agitation and propaganda", was tried in his absence and interned as suffering from "creeping schizophrenia". His release was effected in 1975 following a massive campaign both from the mathematical and psychiatric communities abroad. Speaking in his native Ukrainian, a medium of expression which he explained was itself a symptom of "dissidence" to Soviet forensic psychiatrists, Plyushch declared that he did not wish to recall the details of his incarceration—the massive doses of neuroleptic drugs, the ambience of the genuinely sick and the "doctors" whose

white coats only partially covered their police uniforms.

In an interview with *Nature* he indicated he was concerned rather to campaign for those still in captivity, either his fellow internees in mental institutions or those who, like Vladimir Bukovskii or Semeon Gluzman, are now serving prison sentences for bringing the Soviet abuse of psychiatry to the attention of the world scientific community. Some 1,000 detainees, he estimated, are incarcerated in penal or ordinary mental institutions simply because their views on politics and human rights do not coincide with the official norms. Asked whether he had any regrets in his own case, Plyushch replied that, speaking with hindsight, he could now see that it would have been advisable to proceed more "canily", to be less open and naive, and to make sure that the authorities had less opportunity to gather a dossier against him.

Having acted as a liaison officer between the movement for self-determination in his native Ukraine and the Sakharov human rights committee in Moscow, Plyushch gave some interest-

ing new sidelights on the dissident movement. He stated that the campaign for human rights and civil liberties was primarily a movement of the scientific community, and had as yet little grass-roots following, thus differing sharply from the movement for religious liberty and the "national" movements in the Union (that is, non-Russian) Republics, which included all sections of the community. Only in certain specific areas, notably in West Ukraine (incorporated into the Soviet Union in 1939) is there a close link between the demand of the workers and peasants for better living standards and the campaign of the intellectuals for greater democratisation "on the Czech model".

As a cyberneticist himself, Plyushch stressed that the scientists in the dissident movement must put forward a "new kind of planning" if their ideas are to command the kind of mass support that he sees as the only sure foundation of democratisation.

The kind of union of workers and intellectuals which Plyushch advocates is an important feature of the new protest movement in Poland. Following the protests of workers in the Radom and Ursus plants against rising food prices, a defence committee was

A letter from the USSR

Nature has received this letter, addressed "to all scientists of the world":

Dear colleagues,

By this letter we want to call your attention to the tragic fate of biologist Sergei Kovalev. We want scientists to have a clear realisation of the conditions under which those who venture to protest against arbitrary rule are compelled to live in the Soviet Union. By the nature of their profession scientists strive for adequate cognition of the world. That is why we hope that it will be scientists who gain the best insight into certain features of the Soviet regime. This understanding could be of great importance.

Sergei Kovalev is a gifted scientist, specialist in the field of heart and cell physiology. In December 1974 he was arrested, and in December 1975, after a year of imprisonment, sentenced to seven years of prison camps of severe regime to be followed by three years in exile.

Kovalev's alleged "crime" was that in accordance with his convictions he could not and did not obey an unwritten Soviet law: to keep silent about all acts of arbitrariness and injustice done by Soviet authorities. Kovalev

was one of the first to join the Human Rights Movement. In May 1969 he entered "the Initiative Group" for protection of human rights. At a moment when "The Chronicle of Current Events" (a typewritten journal of political repression in the USSR) was savagely persecuted, Kovalev together with T. Velikanova and T. Khodorovich courageously announced that they would do their best to disseminate this journal since it gives true information about the violations of human rights in the USSR. All his activity in protection of human rights was imbued with noble spirit and humanism.

Sergei Kovalev committed no crime. In any open society he would be one of its most respected members. The Soviet authorities declared Kovalev a dangerous criminal. His imprisonment is not a mere limitation of freedom. In the camps they are trying to "rectify" Kovalev's convictions by isolation, hunger and humiliation. A renowned scientist is forced to do exhausting monotonous physical work, he is limited in correspondence (two letters a month), in visits of close relatives (one visit every four months at most, in practice still less). Malnutri-

tion also is one of the "rectifying" measures; accordingly, during three and a half initial years Kovalev will not be allowed to receive parcels.

But all this does not seem enough. Kovalev is constantly persecuted by the camp administration. He is not given qualified medical treatment and has no possibility of curing a painful chronic disease which makes his prison life unbearable, hard enough as it already is.

We call scientists of the whole world to make use of every opportunity to draw public attention to the tragic fate of Sergei Kovalev.

We call scientists to appeal to Soviet legislative, governmental and party bodies on behalf of Sergei Kovalev.

We call biologists to withhold scientific contacts with the Soviet Union until Sergei Kovalev is released.

ANDREI SAKHAROV, VALENTIN TURCHIN, YURI ORLOV, YURI MNYUKH, NINEL PANFILOVA, YURI GOL'FAND, VINYAMIN LEVICH, GALINA SALOVA, YURI GASTEV, ALEKSANDR LAVUT, TATYANA VELIKANOVA, ALEKSANDR LERNER, TATYANA KHODOROVICH, IGOR MEL'CHUK, ALEKSANDR KORCHAK, VLADIMIR ALBREKHT, NAUM MEIMAN, EFREM YANKELEVICH, ELENA BONNER, YURY SHIKHANOVICH, MARK AZBEL.

set up, including writers, lawyers, historians and scientists. Among those already subjected to reprisals, biochemist Piotr Naumski has already suffered a short term of arrest, while Mirosław Chojewski, a chemist, has been dismissed from his post at the Institute of Nuclear Research in Swierk.

A letter from the Defence Committee to Academician Andrei Sakharov, appealing to his "moral authority and fellow-feeling", evoked a declaration from him that although he did not know what concerted actions could, at the present time, be aligned with the actions of the Polish defence committee, he was nevertheless seeking ways in which the Soviet human rights movement could "effectively cooperate" with similar movements throughout Eastern Europe.

There seems to be enough dissident activity to suggest that the time is ripe for such a concerted effort, if only one of moral support. In the Soviet Union a press campaign has been launched against Aleksandr Voronel, the founder of the illegal "Sunday seminar" for Jewish refuseniks, denouncing him as a traitor. The seminar, which survived Voronel's departure for Israel in December 1974, is now approaching its fifth anniversary, and is planning a

special Jubilee session (Sakharov is to be on the jubilee Committee). Nevertheless pressure on the Seminar continues; recently several of its leading members, including physicist Mark Azbel, the organiser, were held by the police for several days.

In East Germany, the chemist Robert Havemann is subject to house arrest, for having protested against the exiling of Wolf Biermann, the dissident poet and ballad-singer. Havemann's dissident "career" dates back to 1964, when he was dismissed (on account of his "revisionist" views) from his post as Professor of Physical Chemistry at the Humboldt University, Berlin.

From Bulgaria it is understood that an attempt to disseminate some controversial material in *samizdat* form led to the removal of their author (a physicist) to a mental hospital. Although he was subsequently discharged, he was not reinstated in his university post but was instead retired on a disability pension, thereby effectively removing his ability to speak from a position of authority.

This appears to mark a new development. The dissemination of Soviet technology and ideas among the countries of the Socialist block is a basic concept of Comecon. So far, however, the incarceration of dissidents

in mental institutions has been primarily a Soviet phenomenon, although there have been hints of isolated cases in Czechoslovakia and the German Democratic Republic.

● Lord Todd had some words to say last week on the Royal Society's role regarding scientists and human rights. Delivering the Society's Anniversary Address, he said it was hard to see in what way the Society could occupy a special position. He pointed to the Society's adherence to the Declaration issued by the International Council of Scientific Unions and by its cooperation with that body in efforts to uphold it in all its member countries. In appropriate cases, he said,

the Society has drawn and will continue to draw the attention of the Soviet Academy of Sciences or the corresponding body in any other country concerned, as well as our own Government, to the facts and to the need for action with, I believe, good effect. It is my firm belief that the Society as such can achieve much more in this way than it can by subscribing to or issuing public declarations. For it must be recognised that a scientist, as such, is in the same position as any other citizen of his country, subject to the same laws and having the same obligations to the society in which he lives. His profession does not entitle him to special privileges which are denied to his fellow citizens; nor should it deny to him those privileges which are the right of every man. □

WEST GERMANY

Reactor reactions

A Correspondent reports on recent developments on the nuclear power front in the German Federal Republic.

THE future of nuclear power in West Germany depends critically on imminent decisions over the construction of nuclear reprocessing and storage facilities in Lower Saxony. The rumble of anti-nuclear feeling, which recently erupted in demonstrations at the Brokdorf power station site, led the Interior Minister, Werner Maihofer, to announce earlier this year that no new permits for the construction of atomic power stations would be issued until firm plans existed for dealing with spent fuel. The salt mines of Lower Saxony are considered the only suitable site for long-term waste storage. The Prime Minister of Lower Saxony was begged four weeks ago by Bonn Cabinet ministers to allow the plant to be built and seems to have been persuaded: the proposed site of the plant is to be announced soon. But determined local opposition will ensure that its path will not be smooth.

The 20% of Germans against nuclear energy probably entertain strongest fears about reprocessing and storage of nuclear fuels. But the most violent

protests have been not in Lower Saxony but at the site 50 km north-west of Hamburg where Kraftwerk Union is beginning to build West Germany's latest nuclear power station. Brokdorf, on the Elbe, has seen two ugly confrontations between demonstrators and police in the past few weeks, with many injured. The protests are not against any particularly unsavoury feature of Brokdorf but rather against nuclear expansion in general and the way industry is steadily transforming the lower Elbe region into another Ruhr.

The protests could equally well have been focused on Biblis, another Kraftwerk Union product and the world's biggest nuclear plant, which was forced to shut down for three months during the summer after screws had been found dislodged from a cooling pump. An even more controversial aspect of the plant is the intention of the owners, Rheinisch-Westfälische Elektrizitätswerke (RWE), to increase storage capacity for spent fuel pending construction of a reprocessing plant in Germany—RWE apparently find French and British reprocessing too expensive and prefer to sit on their fuel for an extra five or six years until the first German reprocessing plant is in action. Another worry for safety-con-

scious Germans is the prospect of the reactor which the chemical company BASF is to build in an urban area at Ludwigshafen. And a third reactor—that planned for Wyl on the Rhine—is as yet unstarted because of public protest. Even discounting the political element in the demonstrations, it is clear that the new anti-nuclear feeling is not to be taken lightly.

This is recognised by the Free Democratic Party, the junior party in West Germany's coalition government, who have voted at their party conference to put strict limits on new nuclear construction. Though the vote has no binding effect for the government it is thoroughly awkward for them and in particular for the Economics Minister, FDP member Hans Friedrichs, a strong nuclear supporter.

Altogether embarrassing timing for Siemens's announcement of its intention to take on AEG-Telefunken's 50% of Kraftwerk Union, the leading West German power station constructor, thus becoming sole owner. But Siemens is probably the happier of the two concerns. It is confident about the future of nuclear energy and has the resources to see the voracious and currently unprofitable Kraftwerk Union through the next few lean years. AEG-Telefunken, though glad to be rid of what it sees as an albatross, is not getting a particularly good deal. □

IN BRIEF

CERN subscription

At the time of going to press, it seems that the crisis over British international subscriptions, particularly to CERN, has been resolved. The problem arose because the government has been operating a strict cash limits policy, whilst subscriptions to international bodies have grown with the decline in the pound. In the Winter Supplementary Estimates, however, it has been possible to raise the Science cash limits by £2.5 million whilst dropping the University limits by the same amount. This step will not affect any university grants already announced — it

comes from an operational reserve now regarded as more than adequate. The remaining £3.5 million needed to fulfil the subscriptions comes from a variety of sources, the main being a CERN refund of £0.6 million on previous programmes and a re-examination of allowable accounting procedures for research councils in dealing with commissioned research.

Swedes propose nuclear law

New Swedish power stations will not be able to begin operating until they can

demonstrate that reprocessing and disposal of spent fuel can be carried out "in a completely safe manner" if a proposal made by the government last week becomes law in March as expected.

All companies building nuclear power stations would have to specify in detail in what form the spent fuel would be deposited and how the radioactive waste would be transported. The legislation would not affect the six Swedish reactors already in action or being loaded in preparation for action, but would apply to the four at present under construction.

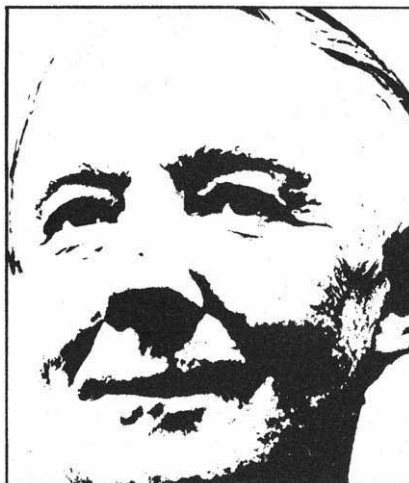
SOME things are done very differently in the United States and in Britain. We are told that Mr Jimmy Carter, the President-elect, will have to appoint some 3,000 people in various offices. Many of those chosen may be rewarded for political services, others may be apolitical and some may even, in the past, have been his opponents. Though Mr Carter will not himself be able to choose each individual personally, he clearly bears a direct responsibility for the whole operation.

In Britain, particularly in the scientific field, changes of government have no immediate effect on the majority of appointments, and there is no wholesale turnover of office holders following a general election. Different governments have made many changes in the pattern of scientific management, generally with unfortunate results, but these have usually been implemented by a process of "general post" with the same faces reappearing with different titles and slightly different responsibilities.

Britain differs most from America in that the way in which many appointments are filled is shrouded in mystery. The full-time senior scientific posts, within government ministries, are mostly filled by the normal civil service machinery, though in many cases the methods by which the office holders are selected are as closely guarded as the register and membership of Cabinet committees. However, the results of this method are generally satisfactory, and sometimes brilliantly successful.

What is even less well understood, however, is the way in which the chairmen and members of influential bodies like our research councils or of the Nature Conservancy Council (whose ancestor was an independent research council until 1965) are selected. Although there are some very square pegs which have been thrust into round holes, in most cases the choice of members has been un-

exceptionable. Some have been active in science and have done distinguished research; a few are still active in their own fields. What is more doubtful is whether they are always the right men (I use the word deliberately, for few are women) to

By appointment**KENNETH MELLANBY**

carry out the duties now apparently expected of them.

It is generally believed that the idea of the independent research council, financed by government, was a good one, and did much to foster high-grade research in Britain. Originally the councils included laymen, some even being politically active members of both Houses of Parliament. The councils' functions were exercised by comparatively infrequent meetings to determine, or more often to approve, major questions of policy, and they interfered little with the general organisation of research and never with the actual work of the scientists they employed. From time to time there were criticisms, such as that even their most senior scientists were excluded from

membership of their own research councils.

I once heard this justified by a member, who said that it would clearly be improper for an employee to be a member of his own governing body. The speaker was a professor much engaged in university affairs; he was temporarily disconcerted when I suggested that his membership of his university's Council and Senate was, by analogy, equally improper. However, most of the active scientists probably preferred the freedom of their laboratories, and seldom hankered after public office.

Today members of these councils seem to be expected to take a much more active part in their work. Many of them are now paid a modest salary which, being honourable men, they feel they must earn. So meetings of the councils themselves, of their committees, working parties and preparatory groups take place interminably. The employed staff, including scientists who should be doing full-time research, spend more and more time "servicing" these meetings (though they are generally excluded from the more important sessions). My general impression is that all this extra work is almost entirely counter-productive, resulting in less and worse rather than more and better research. This is surely what might have been expected. Where research is concerned, no committee has ever had an original idea. The more such committees meet, the more they are likely to obstruct research.

Research councils are now complaining about their shortage of funds. If they ceased to pay their own members and if they met less often this would set free a modest sum to pay a few more bench workers. But more important, the reduction in the time wasted on and by the committees would do much to ensure that a greater proportion of the funds could be spent more fruitfully.

correspondence

Lavoisier's originality

SIR,—Antoine Laurent Lavoisier is generally credited with the independent discovery of oxygen and its role in combustion and respiration. But the existence of his personal copy of the complete works of John Mayow¹ suggests that his ideas were not as original as he claimed. Lavoisier's early detractors seem to have been justified even when they accused him of a lack of originality.

Mayow recognised the existence of oxygen, which he called *Spiritus nitro aereo*, in 1674 when he was experimenting in physiology at Oxford. He described the decrease in the "portion of air that made respiration possible and was essential to life as well as to the combustion of candles". He also described quite accurately the changes occurring in red blood cell pigment exposed to either "fresh" or "corrupted" air. He considered that the portion of air that made it "respirable" was fixed to this coloured compound in the blood cells and acted as an indicator of whether the blood was "respirable" (red, arterial blood) or "corrupted" (dark purplish, venous blood).

There are striking similarities between the writings of Lavoisier² and Mayow on this subject, although the former insisted that he did not know the work of the latter³. Some eminent eighteenth and nineteenth century scholars, however, including Cuvier⁴, maintained that he did, and Hoefer⁵ considered that Mayow was the "real father of modern chemistry and the inventor of the theory of respiration".

Lavoisier seems indeed to have known about Mayow's work because a letter⁶ to him from Mr Koenig of Strasbourg, dated 1767, contained an invoice for 118 books, among them a copy of Mayow's *De Sal Nitro*. As Mayow's tract *De Sal Nitro* was only published in the *Opera Omnia Medico-Physica* one must conclude that this book was the *Opera Omnia*. The book I found recently in the library of the Académie des Sciences de Paris, with the inscription 'De la Bibliothèque de M Lavoisier, Registreur de Poudres et de Salpêtres de France, Inspector Générales du Roy', was one of the 1681 edition of *Opera Omnia*, published in The Hague. This book was in Lavoisier's personal library and then passed on to J. B. Dumas, who died in 1884. Dumas greatly admired Lavoisier and arranged for the publication of his works⁷. In

1937 the book was given to the Académie des Sciences.

Lavoisier, therefore, must have been familiar with Mayow's work when in 1777 he presented his memoirs on oxygen and respiration² to the Académie des Sciences, although in 1776 a letter from M Magalhaes⁷ to Lavoisier suggests that Lavoisier was only then looking for Mayow's works. His subsequent protestation of ignorance³ seems to have represented an eighteenth century version of scientific cheating.

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¹ Mayow, J., *Opera Omnia Medico-physica Tractatibus Quinque Comprehensa* (Leers, the Hague, 1681). See Partington, J. R., *A History of Chemistry*, 2, 582 (Macmillan, London, 1961).

² Lavoisier, A. L., *Mémoires sur la Respiration et la Transpiration des Animaux*, 185 (Académie Royale des Sciences, Paris, 1777).

³ *Oeuvres de Lavoisier*, 7, Letter No. 59 (edit. by Frick, R.) (Editions Albin Michel, Paris, 1955).

⁴ Cuvier, G. L. C., *Histoire des Sciences Naturelles* (Masson, Paris, 1841–1845).

⁵ Hoefer, J. C. F., *Histoire de la Chimie*, 2, Tome II (Hachette, Paris, 1842).

⁶ *Oeuvres de Lavoisier*, 1–6 (edit. by Grimaux, E.) (Imprimerie Imperiale, Paris, 1864).

⁷ *Oeuvres de Lavoisier*, 7, Letter No. 320 (edit. by Frick, R., Daumas, M., and McKie, D.) (Editions Albin Michel, Paris, 1964).

Amazon rain forests

SIR,—The Amazonian rain forests are indeed "one of the world's great photo-synthetic factories", as Thomas Jukes has recently stated (November 11, page 108), but he is wrong to add that they "maintain atmospheric oxygen". The total area of the Amazon basin rain forests is about 3×10^6 km² and the above-ground biomass is on average about 25,000 tonnes km⁻², but this huge mass of vegetation is not a net source of oxygen. Overall it is in equilibrium just as is any substantial area of climax vegetation.

Patches in the building and mature phases of the forest growth cycle do show net increase of fixed carbon and an accompanying release of oxygen, but this is balanced by decomposition of dying vegetation, in which oxygen is taken up, in patches of overmature forest and the following gap phase of the growth cycle. Man-made or natural catastrophes in which large areas are cleared and the vegetation destroyed by burning or natural processes of decomposition do locally and temporarily lead to fixation of oxygen, but these are followed by a vigorous regrowth of vegetation with a rapid build-up of biomass during which oxygen is released.

So the luxuriant regrowth which soon refills a jungle clearing is actually for a time a source of oxygen. What are altered by large scale clearance are the structure and species-composition of the forest, and unnaturally extensive clearance by modern man could progressively deplete the forest of its diversity and lead to reduction in numbers or even extinction of some plants or animals. There is also the possibility of altering reflectivity of the surface, hence the heat balance, hence, conceivably, local climate (Stewart P., *Commonw. For. Rev.*, 55, 155–157; 1976). These are the dangers to be countered as "development" of the rain forest takes place.

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Seveso: putting it right

SIR,—With Alastair Hay's article on Seveso (October 14, page 538) is an item on alternatives to 2,4,5-T in which it is stated that Amcide is made by Nissan in Japan.

Just to put the record straight, Nissan Chemicals manufactures ammonium sulphamate but Amcide is the trade mark of Albright and Wilson Ltd, which markets ammonium sulphamate in the UK.

R. E. C. HAWKINS

Organics Sector,
Albright and Wilson,
Warley, UK

Alastair Hay adds: The article on alternatives to 2,4,5-T also mentions criticisms by environmentalists of the widespread use of polychlorinated biphenyls. In it the phrase "polychlorinated biphenyls (PCBs) such as DDT" should have read "polychlorinated biphenyls (PCBs) as well as DDT". Dichlorodiphenyltrichloroethane (DDT) is not, of course, a polychlorinated biphenyl.

The November 25 issue reported Dr Donald Lee as saying that 130 kg of dioxin (TCDD) was released in the accident at Seveso. Dr Lee has since indicated that his suggestion was that "given the worst possible situations up to 130 kg TCDD could have been produced". His figure does not refer to the quantity of TCDD discharged directly into the atmosphere; as far as he is aware no figures for the amount of TCDD remaining in and around the reaction vessel have been divulged.

news and views

Parity violation in atoms?

from F. E. Close

Is parity violated in atomic physics? According to the experiments being performed independently at Oxford and the University of Washington the answer may well be no (this issue of *Nature*, page 528). This is a very interesting result in light of last month's report (Benvenuti *et al.*, *Phys. Rev. Lett.*, **37**, 1039; 1976) claiming that parity is violated in high energy "neutral current" interactions between neutrinos and matter.

The majority of theoretical high energy physicists currently believe that the weak and electromagnetic interactions are probably unified with the consequence that the atomic physics and high energy neutrino experiments should have shown similar behaviours with respect to parity. Consequently the atomic physicists' observations are highly significant and potentially dramatic for our understanding of Nature. To appreciate their relevance it is worthwhile surveying the developments of the past few years that have set the background for these experiments.

Unification of the weak and electromagnetic interactions

The unification of Nature's forces has long been the aim of physicists. In the 19th century Maxwell unified electricity and magnetism and in the past few years there has been an increasing belief among physicists that the familiar electromagnetic interaction may well be unified with the weak interaction. This latter mysterious force is responsible for β decay and is intimately linked with parity violation. If this unification idea is correct, then it would rank as one of the major advances in our understanding and hence much effort has been directed towards investigating it.

On the theoretical front there is the unified model of Weinberg (*Phys. Rev. Lett.*, **19**, 1264; 1967) and Salam (in *Elementary Particle Physics*, edit. by Svartholm N., 367, Stockholm, 1968). This idea was attractive but appeared to suffer from the difficulty that at high energies certain processes had infinite

probability. In the past few years there has been an upsurge of interest in this model as a result of three discoveries each of the first order of magnitude in significance.

In 1971 G. 't Hooft of the University of Utrecht showed that the theory was renormalisable, that is the infinities are no different from those that we are used to meeting in quantum electrodynamics and can be removed by traditional tools. This meant that for the first time one had a viable theory of weak interactions which did not violate any known general principle such as relativity, causality and unitarity and which, furthermore, suggested an intimate relation between weak and electromagnetic interactions.

On the experimental side was the discovery of "neutral current" interactions in which a neutrino interacts with matter without transfer of electrical charge, in contrast to the previously well known "charged current" interaction where the neutrino turns into a charged lepton (e^- or μ^-) with associated transfer of electrical charge to the target. That such neutral currents should exist was predicted in the Weinberg-Salam model and so their discovery was greeted with excitement (*Nature*, **245**, 119; 1973; **249**, 211 and **250**, 186; 1974). The charged current interactions have been known for many years to violate parity. The model predicted that the neutral currents should also be parity violating and this was confirmed by neutrino experiments only last month (Benvenuti, *op cit.*).

The discovery of neutral currents raised a problem, namely why is strangeness conserved in their interactions, in contrast to the charged current case? The theoretical answer was to postulate that a new family of particles should exist with a property called charm and with rather distinctive production and decay behaviours which have the effect of cancelling the unwanted strangeness-changing neutral currents (see *Nature*, **259**, 622; 1976). Two years ago this month came the

momentous discovery of the J/ψ particle (see *Nature*, **252**, 438, and **254**, 387; 1975) which led to the Nobel Prize for physics being awarded jointly to B. Richter and S. Ting just a few weeks ago. This discovery was like finding a previously hidden window and the view through it led to the discovery this summer of the charmed particles. Consequently all the phenomena required for the unified theory to be valid were indeed being seen. Furthermore, by July of this year, a wide variety of data was being reported at the International Conference in Tbilisi USSR (see *Nature*, **262**, 537; 1976) in accord with the original unification idea of Salam and Weinberg.

Put all of these discoveries together and the excitement about unified theories of weak and electromagnetic interactions is natural.

Parity violation in atoms

According to the Weinberg-Salam model one should see parity-violating effects not only in $\nu \rightarrow \nu$ but also in processes involving electrons ($e^- \rightarrow e^-$) or muons. In these latter cases the parity-violating weak neutral current is swamped by the parity-conserving electromagnetic current; hence one would have to look for subtle effects. One such effect would be that a parity forbidden electric dipole transition could take place between atomic energy levels such as $1S_1$ and $2S_1$, in addition to the parity allowed magnetic dipole transition. It is effects associated with such small "wrong parity" admixtures (see Brodsky, S. and Karl, G., *Parity violation in atoms*, in *Comments on Atomic and Molecular Physics*, **5**, 63-69; 1976) that are being looked for by Sandar *et al.*

As these experiments currently stand, the predictions of the Weinberg-Salam model supplemented by atomic physics calculations appear inconsistent with the data.

As stated by both groups, their results are consistent with no parity violation. At present the discrepancy can conceivably be the combined effect

of systematic errors in atomic physics calculations and systematic uncertainties in the experiments. If, however, this state of affairs persists to a factor of ten below the present level and future high energy neutrino experiments (such as those about to begin at the CERN SPS in Geneva) continue to uphold the parity violation recently reported, then a rather paradoxical situation will have arisen in the light of currently favoured ideas.

The conclusion would probably be that neutrino interactions violate parity both when charge is transferred or when the neutral current process occurs. The parity-violating neutral

current with neutrino beams would have no obvious relation with electron beams which conserve parity. This is quite a tenable viewpoint—if one doesn't believe in unified theories! Alternatively, one might argue that there is some strange energy dependence whereby the high energy experiments show parity violation which is less marked at low energies (as in atomic physics). Whether such a possibility could be incorporated into the unification ideas is not clear. It also isn't clear, yet, if we have to worry. However, the clear blue sky of summer now has a cloud in it. We wait to see if it heralds a storm. □

about 30 tons is similarly driven through a 5.6 m rack and worm.

The azimuth and elevation positions are read into the computer from digital systems on the telescope axes and conventional drive systems give following accuracies on sidereal coordinates fed into the computer to ± 0.3 arc s. In actual operation the final guidance is by manual or automatic photoelectric corrections, on the object or offset star. These outputs are both fed into the computer and also operate quartz plates which adjust the position of the image. Experience has shown that the operational accuracy is one-fifth of the diameter of the image. That is, if the seeing disk is 1 arc s the guidance is accurate to 0.2 arc s. At the prime focus the manual setting system only is available but both manual and photoelectric devices are available at the Nasmyth foci. The controversial question of the problem of correction for image rotation in an alt-azimuth telescope appears to have been solved satisfactorily by these arrangements. In a 20 min exposure the errors in the correction for field rotation do not exceed 0.3 arc s. The slewing speed of the telescope is 45 deg min^{-1} in azimuth and 30 deg min^{-1} in elevation.

The finder telescope is of 70 cm aperture and objects of 17 mag are visible on the television display. Two fields of view, 30 arc min or 11 arc min, are available. At the prime focus guidance can be made directly or by offset on a 19 mag object. A television system for use at the prime focus is under development and it is expected that this will enable direct guidance to be carried out on objects to a limiting magnitude of +21.5 in blue colour.

The mirror

The 6 m mirror is of Pyrex glass. During the casting of the blank two small bubbles appeared and these spread somewhat during the grinding process. Small baffles in the region of the prime focus blank out these regions, but the consequent loss in intensity is only 10%. Otherwise the mirror has fulfilled expectations and although it will be replaced eventually by a mirror of SITALL material (the Soviet equivalent of CERVIT) there is understandably no great anxiety about the probable delay. The position on the availability of Soviet mirror blanks in SITALL is that blanks of 2 m are now available and the 6 m blank is expected to be available between 1979 and 1980 and the probable date for installation in the telescope is thought to be about 1983. The focal length of the 6 m mirror is 24 m giving a field of view at the prime focus of 2 arc min which can be increased to 20 arc min by the field correcting plates. The operating posi-

The Soviet 6 m telescope

from Bernard Lovell

It has been difficult in the West to obtain consistent information about the state of the Soviet 6 m optical telescope. It was therefore with particular interest that I received an invitation from the Soviet Academy of Sciences to visit the recently created Astrophysical Observatory, in the North Caucasus. The principal instruments of this observatory, which has the status of an Institute of the Academy, are the 600 m RATAN radio telescope and the 6 m optical telescope. A recent account of the RATAN radio telescope has been published (*Nature*, **262**, 535, 1976) and the following account deals mainly with the 6 m telescope as it existed in mid-October 1976 when I visited the observatory and talked with Dr I. M. Kopylov the director, Dr B. K. Ioannisianni the chief designer and several members of the observatory staff.

Before choosing the site, I was told, fifteen areas in the Soviet Union were tested. It seems that an area in the Asian Republics might have been chosen but for the difficulty of transportation and constructing the telescope in such a region, and eventually the North Caucasus region was favoured. The telescope is 250 km from the airport of Mineralniye Vody, a 1 h 45 min jet flight from Moscow. The route rises slowly until the small town of Zelenchukskaya is reached 210 km from the airport and on the outskirts of this town the RATAN radio telescope has been constructed at an altitude of 1,000 m. From Zelenchukskaya the road continues normally for a further 24 km to where the new scientific village of Nizniy Azkhyz is

under construction. This village at present consists of a block of 70 flats for the scientists, a kindergarten, two shops and a cinema. The main scientific laboratory is under construction and more schools, a hotel and restaurant for visiting scientists are planned. From here an entirely new road 17 km long has been constructed to the telescope site which is at an altitude of 2,100 m in Stavropol territory, latitude $44^{\circ}44'N$, longitude $02^{\circ}40'E$. A small hostel in which we were housed provides a pleasant residence in beautiful surroundings about 800 m from the telescope. When the hotel in the village is complete this hostel will be used only by those using the telescope.

Design and guidance

The design of the telescope is unique in that it is the first large optical telescope to make the break from the conventional polar axis to an alt-azimuth mount. This has resulted in a most elegant construction and the system is clearly a great success. The rotating part of the telescope weighs 800 tons carried on six oil pad bearings and a locating but not load-bearing pedestal beneath. The vertical is maintained to 2 arc s by this system. The azimuth drive is through a 5.6 m gear and worm; the 5.6 m rack with 512 teeth was cut in one piece. This precision worm drive is protected from accidental overload by a spur gear and pinion, mounted immediately beneath it. If the load on the main drive rack reaches a specified level the worm is automatically shifted laterally so that the overload is taken on the subsidiary system. The elevation cage weighing

tions are either at the prime focus or at the two Nasmyth foci. The image scale is $0.12 \text{ mm arc s}^{-1}$ at the prime focus and $0.90 \text{ mm arc s}^{-1}$ at the Nasmyth foci.

Recording systems

In addition to direct photography two spectrographic cameras are available at the prime focus. Three gratings of 200, 300 and 600 lines mm^{-1} and a selection of optical systems give dispersions in the range 60 to 370 $\text{\AA mm}^{-1}$. One Nasmyth focus is occupied by equipment for stellar spectroscopy. The other focus houses an echelle spectrograph with two gratings. One with 100 lines mm^{-1} giving a dispersion of 36 $\text{\AA mm}^{-1}$ in the range 3,200 to 6,700 $\text{\AA}$, and the second with 50 lines mm^{-1} giving a dispersion of 45 $\text{\AA mm}^{-1}$ in the range 6,500 to 11,000 $\text{\AA}$. The modern Kodak emulsions are used and up-to-date emulsion treatment facilities are available.

As regards electronic recording systems a one to three stage image tube system is working in the laboratory and will be tested at the prime focus before the end of November. Initially the spectrum will be recorded photographically but I was shown a digital recording system in an advanced state of development in the laboratory and was informed that this would be in use on the telescope in 1977. In this system real time sky subtraction will be available.

Sky conditions and sensitivity

The site has been carefully chosen to provide excellent seeing conditions. From the limited time for which the telescope has been in use the opinion is that 100 nights per year are absolutely clear and 200 nights about half clear, and for about 20% of the time the seeing conditions are 1 arc s. It is believed that temperature effects on the Pyrex mirror contribute significantly to this disk and that this 20% figure may be reduced to 0.5 arc s when the SITALL mirror is available. The present sensitivity of the system is that objects of +24 mag in blue colour with a signal-to-noise ratio of 3 to 5 are obtained with exposures of 30 to 40 min. Objects of magnitude +25 in blue appear with these exposures with a 64% probability of identification. In red the limit is +23 to +23.5 magnitude for a 90-min exposure. Dr Yu. Karovjakovsuy kindly presented me with a recent photograph of NGC 2419 obtained in blue colour with a 40 min exposure in which stars of +24 magnitude are clearly visible.

Contrary to the system favoured at Kitt Peak and Siding Spring the dome is effectively at ground level. Naturally the rotation is controlled by the telescope drive computer and the con-

struction, offices, laboratories and general facilities are of the highest quality. The telescope is already being visited by a considerable number of groups of the public. They are shown a film of the construction of the telescope in a lecture theatre inside the dome and can inspect the telescope through a viewing gallery. The telescope area itself is air conditioned and temperature controlled. The temperature is set to the predicted night temperature some hours before observations commence.

The chief designer of the telescope, Dr Ioannisiani, told me that the cost of the telescope itself together with associated equipment was about 30 million roubles. At current exchange rates this is of the order \$36 million, although it must be remembered that because of large variations in the cost of labour and materials the equivalence must be treated with caution. The cost of the dome, of the road up the mountain, the buildings at the observatory site and the erection of the new village, in addition to the cost

of the RATAN radio telescope implies that the Soviet Academy has invested a substantial sum of money in this new Institute. This courageous enterprise has given Soviet astronomers an unsurpassed instrument for the study of the heavens. The final tests and adjustments of the telescope by the observatory staff will be completed shortly and from January 1977 it is expected that the telescope will be in regular use for astronomical research. The instrument probably marks an historical epoch in the development of astronomy for two reasons, first because it is unlikely that a larger telescope will ever be constructed on Earth, and second because the success of the alt-azimuth mounting may lead to the abandonment of the polar axis design for any future large telescopes.

I wish to express my gratitude to the Academy of Sciences of the USSR for the invitation to visit this telescope and to Dr Ioannisiani, Dr Kopylov and many members of the observatory staff for their kind attention during my visit. □

Rethinking human evolution

from our Palaeoanthropology Correspondent

MANY areas of science occasionally make rapid advances when new data, new analytical tools and new areas of investigation suddenly open up new realms of perception, insight and knowledge. During such a phase the accumulation of data may proceed so rapidly that the older theoretical foundations to which these data are applied may require rethinking and revision. When that occurs it is necessary to step aside briefly in order to reassess those theoretical frameworks from the vantage of the new evidence. The study of fossil man has recently been undergoing such a period of saltatory expansion. The abundant new African material, from Lake Turkana (formerly Lake Rudolf), Laetolil and Hadar has accumulated so rapidly that there has been little time to reflect on how this material may affect current theories of evolution and our understanding of certain taxonomic categories.

Within the early years of this decade students of human evolution have already had to recognise two important and inter-related alterations in earlier theories of human emergence. The first of these concerned the evidence that two types of hominid had existed contemporaneously within Plio-Pleistocene times. Although some work had earlier

indicated such sympatry, the Lake Turkana and Hadar discoveries have provided virtually unquestionable evidence that at least two forms of hominid had coexisted (see Leakey and Walker, *Nature*, **261**, 572; 1976).

Such evidence indicates that the earlier and simpler models of "straight-line" evolution (for example, *Ramapithecus* → *Australopithecus* → *Homo*) could not be substantiated in the fossil record. Thus, the evolution of the Hominidae is more complex, and ultimately more interesting, than previous indications.

The second implication of the new fossil material is that the genus *Homo* may be far older than previously thought. A recent paper in *Nature* (M. Leakey, *et al.*, **262**, 460; 1976) suggests the presence of *Homo* at Laetolil, Tanzania as early as 3.77 Myr ago. In that suggestion the authors have demonstrated, perhaps unwittingly, one of the more serious problems in studies of early man today. That problem is a lack of usable and valid definitions for genera within the family Hominidae. In that paper, the authors point out certain similarities between the jaws and teeth of the Laetolil hominids and others from such sites as Olduvai, Sterkfontein and Taung. Yet the Sterkfontein hominids have often been

placed within the gracile australopithecine group and Tobias has favoured placing the Taung child within the robust australopithecines. The acknowledgement of such morphological affinities does not strengthen the final attribution of the Laetoli hominids to the genus *Homo*.

However, the suggestion that this, or any material, belongs to the genus *Homo* should not rest solely on the presence of certain dental features. All hominids share a basic, underlying morphological pattern which is particularly evident in the jaws and teeth. The ultimate placement of fossils in the genus *Homo* must be based, first, on a clear statement of what characters constitute that genus and how it is distinguished from other contemporaneous hominid taxa.

Walker has recently (*Earliest Man and Environments in the Lake Rudolf Basin*, edit. by Coppens, *et al.*, Chicago University Press, 1976) taken a step in this direction by proposing a working model for a differential diagnosis between the Plio-Pleistocene hominid genera. Admittedly tentative, this model is of interest in that it focuses on a broad spectrum of cranial and facial features but avoids use of specific dental characters. Such a model could provide a useful point of departure for a broader and widely acceptable definition of early hominid genera. Ultimately, however, such a definition must also be based on character complexes of the post-cranium. For example, we are now in a position to begin discussing limb proportions, weight distributions in the lower limb and the structure of the hip and knee joints in the early hominids.

Walker's model also provides a warning concerning the problems inherent in basing attributions on single characters or on a tightly inter-dependent suite of characters. When the cranium of KNM ER 1470 was found in 1972 it was widely considered to have belonged to an early member of the genus *Homo*. This attribution was based almost entirely on its relatively large cranial capacity (about 775 cc) and on skull characters related to that size. Walker argues, however, that when the absolute size factor is de-emphasised the total morphology of this skull does not demonstrate clear affinities with *Homo*. Although Walker stops short of attributing the KNM ER 1470 cranium to *Australopithecus*, its position within *Homo* is clearly now uncertain. The taxonomic position of the Laetoli hominids must also be considered uncertain. Both, however, provide useful examples of some of the intrinsic problems in our current poorly defined taxonomic schemes. The large amount of newly available data should, hopefully, be put to good use in improving

our recognition of the distinctions between the early hominid groups. □

Mathematics and casinos

from Robert M. May

JAMES BOND fans will recall the climactic moment of the first book, *Casino Royale*, when the fate of Bond's mission hung on a single hand of chemin-de-fer. This two-person zero-sum game is one of several variants of the generic game baccarat, in which each player is dealt two face-down cards, and has the option to add one face-up card (the banker deciding last). The aim is to maximise the point count of the hand, modulo 10, with court cards counting as zero; thus 9 is the best count. The game is mathematically interesting in that non-trivial game theory concepts are appropriate. The complete minimax solution for chemin-de-fer with an infinite deck (or, equivalently, a finite deck sampled with replacement) was given by Kemeny and Snell (*Am. Math. Monthly*, **64**, 465; 1957), and shown by Karlin to be an example of a general result (*Mathematical Methods in Games, Programming and Economics*, Pergamon, London, 1959). The player's optimal strategy is deterministic in all cases but one: when his first two cards total 5, he should draw with probability 9/11 and stand with probability 2/11. The banker likewise acts deterministically unless his count is 6 and his opponent has not taken a third card, whereupon he draws with probability 859/2288 and stands with probability 1429/2288. When both players adhere to their minimax strategy, the banker has an average gain of 1.28% of the stake. In the heart-stopping hand, Le Chiffre held a count of 3, having just dealt Bond an exposed (third) 9 card, and both players agonised over his decision whether to draw a third card. As this was in 1953 or earlier, their ignorance may be pardoned. Kemeny and Snell's exact optimal strategy mandates that Le Chiffre draw (as he eventually did).

Before July 1, 1970, the British Gaming Act covering banker's games permitted any game so long as the "chances" in it were equally favourable to all players, including the banker. This fatuous legislation initiated some fascinating statistical studies (Downton, *J. R. Statist. Soc. A* **132**, 543; 1969; Downton and Holder, *loc. cit.*, **135**, 336; 1972; Holder and Downton, *loc. cit.*, **135**, 221; 1972; Downton and Lockwood, *loc. cit.*, **138**, 228; 1975 and **139**, in the press; 1976), as the "chances"

generated by games like baccarat and blackjack with finite decks are not readily amenable even to sophisticated mathematics in conjunction with fast computers. The new Gaming Act is more sensible. It allows four games (roulette, dice, baccarat, blackjack) to be played in licensed clubs, and defines the rules of play.

Downton and his colleagues have explored the mathematical properties of the four permitted games. Roulette and dice are games of pure chance, and are relatively simple to analyse, although some interesting features emerge (of which more below). For baccarat in its chemin-de-fer version, the banker's expected gain of 1.28% under optimal play with an infinite deck is independent of the player's strategy (Downton and Holder, *op. cit.*), so that this game is also effectively one of pure chance for the player. The use of a finite deck makes for vastly greater mathematical complexity (Downton and Lockwood, *op. cit.*). Depending on the size of the deck, measured in units of 52 card packs, and on the cards that have gone before, there are situations where the banker should randomise his decision when holding 3 and giving 8, holding 4 and giving 1, holding 5 and giving 4, holding 6 and giving 6, as well as the Kemeny and Snell case of holding 6 and giving no card (but Le Chiffre should still always draw when holding 3 and giving 9). The odds are but little affected by all the complications (the banker's expected gain with a three-pack deck is 1.31%), and Kemeny and Snell's strategy remains an excellent approximation with finite decks.

In blackjack the aim is to get close to a count of 21 (court cards counting as 10) without exceeding it. Subtle variations in the rules make for interesting differences in the player's strategies and in the odds (see the papers by Downton and Holder). The variant played in American casinos attracted much attention in the 1960s, when the perfect player was shown to enjoy an advantage of about 0.1% with a one-pack deck (Thorp, *Proc. natn. Acad. Sci. U.S.A.*, **47**, 110; 1961; *Rev. Int. Stat. Inst.*, **37**, 273; 1969; Epstein, *Theory of Gambling and Statistical Logic*; Academic Press, London, 1967). With a four-pack deck, the odds favour the bank. The version permitted in Britain under the current Gaming Act gives the bank a gain of 0.13% and 0.59% with one- and four-pack decks respectively. Casino pontoon, the variant played in most British midland clubs in the 1960s under the old Gaming Act, gives the banker an edge of 1.57% and 0.63% with one- and four-pack decks: notice here that, unlike blackjack, the bank's advantage de-

creases with increasing deck size. It is amusing that, misguided by the American experience, most midland clubs played with four-pack decks, thereby diminishing their advantage. Of course few players have the skill to achieve these odds, and the bank typically wins around 2–15% of the stake per hand. Downton has verified these figures in an extended series of undergraduate laboratory experiments.

This work has intrinsic interest. It can also be tortured into yielding two morals for ecologists. Downton notes that an interesting characteristic of any game of chance is the "gain ratio", defined as the bank's average gain per unit stake divided by the standard deviation: the gain ratio is the reciprocal of the coefficient of variation. Although all players are doomed to long-term losses, the smaller the gain ratio the higher the probability that a player will win (or, equivalently, will survive through) any one session. In roulette, gain ratios vary from 0.46% playing single members to 3.77% playing groups of 24; in dice or craps they range from 0.99 to 4.42%; in chemin-de-fer with best play the gain ratio is 1.34%. The significance of the gain ratio is illustrated by considering a casino which spins its roulette wheel 90 times per hour. Suppose 20 people each bet £1 on each spin over an 8-hour session. The bank's expected winnings are £390, but the standard deviation is £189 if all players bet on groups of 12 numbers, £706 if all bet on single numbers. This is a consequential difference. The processes whereby, in Reddingius's memorable phrase (*Oecologia*, 5, 240; 1970), plants and animals "gamble for existence" in an unpredictable world has many similarities with these games. The notion of "gain ratio" is likely to have fruitful applications in ecology.

Casinos and gamblers are usually ignorant of the mathematical structure of their games and strategies, which have, rather, evolved by an intense and highly motivated process of trial and error. In some respects they therefore invite analogies with biological processes of evolution by natural selection. This is particularly so for the way primitive societies may, or may not, have unconsciously "evolved" optimum strategies of resource management. The advantage of gambling games is that the empirically evolved strategies can be compared with the exact optimum ones. Chemin-de-fer provides a beautiful example: the optimum strategy is for the banker to vary his choice of drawing or standing only when he holds 6 and has given no card; most clubs have mandatory instructions to the dealer, and these used to be Crockford's drawing card which

allowed options in three cases, namely, holding 6 and giving no card, holding 5 and giving 4, and holding 3 and giving 9; since 1972 the instructions provided by the Casino Association of Great Britain allow the banker a choice in the latter two cases only. Although the differences are inconsequential, it is notable that the strategies chosen by the casinos were never the best, and have changed for the worse. Casino owners should read Downton's work. □

Surprises in the Magellanic Clouds

from M. G. Edmunds

THE research potential of the Magellanic Clouds—the two nearby galaxies easily visible to the naked eye in the Southern Hemisphere—is underlined by the surprising result of a recent investigation of a star cluster in the larger of the two Clouds. An Australian group (Gascoigne *et al.*, *Astrophys. J.*, 209, L25; 1976) have used the Anglo-Australian 3.9-m telescope to study NGC2209, one of the so-called "intermediate age" globular star clusters in the Clouds.

For stars it is notoriously difficult to assign a reliable age by appearance. But for a cluster of stars, the morphology of a plot of colours of the stars against intrinsic brightness can yield a fair guess. The method essentially works by looking for the highest mass (that is, brightest and bluest) dwarf stars which are only just beginning to evolve into giants. This "turn-off point" from the Main Sequence of dwarf stars can then be compared with computed models of stellar evolution to estimate an age.

In physical appearance a globular cluster has a characteristic spherical shape, held together by the mutual gravitational interaction of the stars. All globular clusters in our own Galaxy seem to have formed at the same time as, or not very much later than, the formation of the Galaxy itself—they are "old" objects. In the Magellanic Clouds there exist globular clusters which have almost certainly formed recently, as evidenced by the existence of bright young blue dwarfs which will live only a short time as dwarfs on the Main Sequence. The "intermediate age" clusters formed sometime between the young and old. Since there are only old clusters in our own Galaxy, these young and intermediate age Magellanic Cloud objects are of great interest. What is surprising is that the Aus-

tralian group find good evidence that the stars in the NGC2209 cluster have relatively few heavy elements ("metals") in them, only about one-sixteenth of the solar abundances, and yet they estimate the age of the cluster as a mere 8×10^8 yr compared with a probable age in excess of 10^{10} yr for the Large Cloud itself. The problem is that the interstellar medium in the main body of the Large Cloud, as determined by several independent investigations of the gaseous nebulae, is certainly not less than one-half solar. If the cluster were very much older, then its composition could be explained by saying it formed out of metal-poor gas, and that metal synthesis in the supernovae explosion of massive stars had enriched the interstellar medium since then. But here is a cluster which is apparently fairly recently formed, and should therefore reflect the present chemical composition of its precursor interstellar medium. It is true that the cluster is one of the most outlying—some 6° or 6 kpc away from the centre of the main body of the Large Cloud, but it is unlikely that the chemical composition of the interstellar medium varies so drastically across the Cloud. Perhaps an outlying cloud of gas somehow survived with very little star formation and consequent metal enrichment, until efficient star formation was suddenly induced.

The metal abundance of the cluster seems well demonstrated since the group used two independent methods. One method relies on the displacement of the locus of stars (relative to Galactic solar composition star clusters) on the colour/brightness plot, due to the weakness of absorption by metal lines in the blue spectral region. A second estimate comes from taking advantage of the light-gathering capacity of a large telescope and isolating just a few spectral lines in two of the stars by interference filters. A comparison of the line strength with that observed for stars in clusters of known composition yields a metal abundance estimate. Both methods agree that the cluster is considerably metal deficient.

The accurate aging of the cluster is more uncertain. The authors unfortunately do not quote error limits on their estimate. An inspection of their published data suggests that using their aging method, errors would not push the age back further than 2×10^9 yr—still young compared with old clusters and the probable age of the Clouds.

If the age of the cluster is confirmed in subsequent work, then considerable revision of our ideas about the chemical homogeneity of the interstellar medium in galaxies may be required. Investigation of other similar clusters in the Clouds will be awaited with interest. □

Salt tolerance in halophytes

from C. D. Field

In large areas of the world, the soil is too saline to support economic agriculture and more land becomes non-productive each year because of salt accumulation. So recent research into the ability of halophilic plants to survive in very saline environments has taken on a new importance.

The basis of the salt tolerance of halophilic plants is their ability to maintain and tolerate a high salt concentration in the cell. Since many halophytes show a positive growth response to salt in their environment (although they may also survive in a salt-free environment) it has been argued that as well as participating in the necessary osmotic adjustments, the high ion concentration may also be required by the plant for normal metabolic activity.

One possibility might be that the enzymes of halophilic plants, like those in salt-tolerant bacteria, require high salt concentrations for optimal activity. But this possibility seems to have been disproved by recent work (see Flowers, *Ion Transport in Plant Cells and*

Tissues, North Holland; 1975) which shows that *in vitro*, enzymes from halophytes are inhibited by ion concentrations much lower than those expected to occur in the cytoplasm, and very similar to those tolerated by enzymes from non-halophytes. Flowers has recently shown (*Proc. R. Soc. Lond.*, **B273**, 523; 1976) that malate dehydrogenase from the halophyte, *Suaeda maritima*, is activated at electrolyte concentrations similar to those required to activate the enzyme from non-halophytes. All the evidence therefore points to the conclusion that halophyte enzymes are neither particularly salt resistant nor salt requiring.

This leads to the idea that the excess sodium ions must be isolated from the cytoplasm in some way. Indirect evidence from efflux analysis and ion mobilities in tissues of halophytes suggests that sodium ions are retained in the vacuole (Flowers, *op. cit.*) and Jeschke and Stelter (*Planta*, **128**, 107; 1976) have shown that sodium ions are largely excluded from the cytoplasm of the halophyte *Atriplex* and barley, using flameless atomic absorption spectroscopy on thin slices of single roots.

This however raises enormous problems of osmotic imbalance between the

cytoplasm and the vacuole, which on this hypothesis would hold most of the salt accumulated in the cell. This osmotic imbalance could only be overcome if the water potential of the cytoplasm could be lowered, so that it will attract water from the vacuole and remain hydrated. This could theoretically be achieved in various ways. The cytoplasm might have an unusually high water-holding capacity arising from its structure, Donnan equilibria might exist between cytoplasm and vacuole, or a non-toxic osmoticum might accumulate in the cytoplasm. There is little evidence for the first two possibilities, but there is increasing support for the third.

Some years ago Stewart and Lee (*Planta*, **120**, 279; 1974) proposed the amino acid proline as the possible osmoticum. They found that in many halophytes proline accounts for more than 30% of the amino acid pool, whereas it is much less in glycophytes although the level in some species increases in response to salt or water stress. Stewart and Lee also showed that proline concentrations of up to 600 mol m^{-3} do not inhibit enzyme activity *in vitro*.

At a recent International Workshop on transmembrane ionic exchanges in plants (CNRS, Rouen, July 1976) Wyn Jones *et al.* put forward another candidate, the quaternary ammonium compound betaine, for the balancing osmoticum. They demonstrated that the betaine content of the halophytes *Atriplex spongiosa*, *Spartina x townsendii* and *Suaeda monoica* increased with external sodium chloride concentration, and that there was a close correlation between betaine accumulation and an increase in sap osmotic pressure above about 400 osmol m^{-3} . They also found that the *in vitro* activity of malate dehydrogenase was not inhibited by 1 M betaine, and that in the presence of low malate concentrations betaine provided partial protection to the enzyme against potassium chloride and sodium chloride toxicity.

These results suggest that betaine may have a similar role to proline in the cytoplasm of certain halophytes. Flowers has recently confirmed that similar levels of betaine occur in the halophyte *Suaeda maritima*. But in all cases direct experimental demonstration of betaine in the cytoplasm is still needed.

A simplified picture of a typical halophilic plant cell now emerges in which the potassium ion and sodium ion concentrations in the cytoplasm are of the order of 100 mol m^{-3} and 150 mol m^{-3} , whereas the vacuole has corresponding concentrations of 100 mol m^{-3} and 500 mol m^{-3} (Flowers *op. cit.*), and the osmotic balance in the cytoplasm is preserved by proline

Hormones, ducks and sex

from John Krebs

It is well known that steroid hormones influence sexual and aggressive behaviour in many vertebrates. These established and documented effects are of a rather long term nature, involving changes over developmental, seasonal and similar time periods. Recently, J. Balthazart (*J. Zool.*, **180**, 155; 1976) has suggested that much shorter term changes in behaviour might also be caused by fluctuations in hormone levels. He recorded the daily pattern of behavioural activities in two groups of five male domestic ducks housed in outdoor cages during winter. At the same time he took serial blood samples from each male to measure plasma testosterone.

The most interesting behaviour categories for the present discussion were courtship displays, mating attempts, and aggressive attacks on other males or females (there were two females in each group of males). Courtship and mating activity decreased through the day after an early morning peak, although some of the other activities did not show a similar daily rhythm, so there was not simply an increase in lethargy as the day progressed. In relating

these changes to plasma testosterone, Balthazart found that the hormone was at a higher level early in the morning than at other times of day, correlating well with the frequency of sexual behaviour. Further, some of the contrasts between the two groups were associated with hormonal differences. One group showed an increase in both display frequency and hormone levels in the afternoon while the other showed neither, but this second group had a higher level of sexual activity overall and a higher mean level of testosterone.

Behavioural differences between individual males did not relate clearly to hormone levels. There was a very slight tendency for males with higher testosterone levels to be more sexually active, but differences in daily rhythm did not relate to hormonal differences.

Balthazart's results raise an intriguing explanation for a mechanism of short term changes in behavioural activity, but it still has to be shown that the changes in hormone level cause the behavioural changes, rather than the other way round.

or betaine.

As with most models anomalies inevitably arise and in this case some of the marine algae raise pertinent questions. In *Valonia ventricosa* the cytoplasmic potassium level has been reported as of the order of 440 mol m^{-3} and the sodium level as about 40 mol m^{-3} whereas the corresponding vacuolar levels are about 620 mol m^{-3} and 40 mol m^{-3} (Gutknecht, *Biol. Bull.*, **130**, 331; 1966). The cytoplasmic potassium concentration in this instance is well above the demonstrated level for inhibition of enzymes from other halophytes. A re-examination of the ion concentrations in the various compartments of this particular alga is clearly indicated. At the same time it would be useful to find whether any metabolic adaptation such as adaptation of enzymes to salt has occurred or whether any compatible organic solutes are present in unexpectedly high quantities.

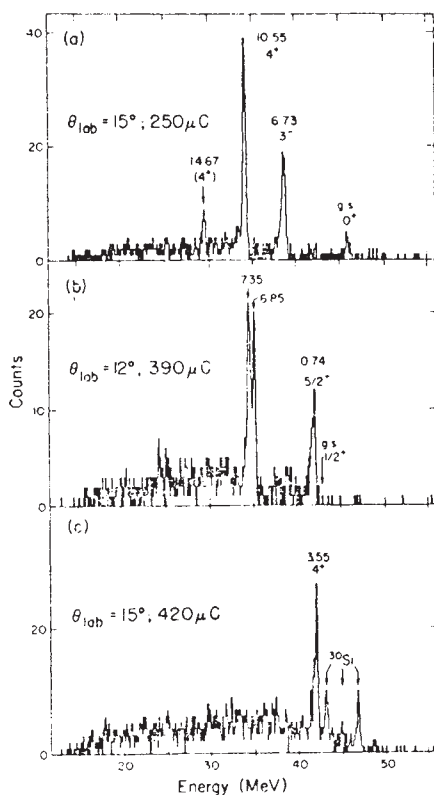
Nuclear spectroscopy with the $(\alpha, {}^2\text{He})$ reaction

from P. E. Hodgson

NUCLEON transfer reactions are one of the most powerful ways of determining the properties of nuclear states, and over the years very many studies have been made of practically every nucleus in the periodic table. The one-nucleon transfer reactions are particularly useful for determining the single-particle properties of nuclei, while reactions transferring more than one nucleon provide information about their collective properties.

For practical reasons not all nucleon transfer reactions are equally easy to carry out. For example, reactions with incoming or outgoing neutrons cannot be studied with the same energy resolution as those involving charged particles. Any addition to the list of reactions available for nuclear spectroscopy is therefore most welcome, and in recent years use has been made of reactions that have unstable outgoing particles.

One of the first of these unstable particles was the nucleus ${}^8\text{Be}$, which rapidly decays into two alpha particles. The energy of breakup is however very small, only 96 keV, so the two alpha particles are emitted in almost the same direction, and are thus easily detected. Now another such particle, the nucleus ${}^2\text{He}$, consisting of two protons in an ${}^1\text{S}_0$, $T=1$ state, has been shown by a group at Berkeley to be detectable in nuclear reactions. This is important because the $(\alpha, {}^2\text{He})$ reaction can now be studied, and this provides a convenient



Energy spectra of ${}^2\text{He}$ nuclei from the $(\alpha, {}^2\text{He})$ reaction on ${}^{12}\text{C}$ (a), ${}^{13}\text{C}$ (b), and ${}^{16}\text{O}$ (c).

way of transferring two neutrons to a nucleus.

In their preliminary studies (*Phys. Rev. Lett.*, **37**, 812; 1976) they show how the ${}^2\text{He}$ nuclei can be detected by a pair of proton counters. Almost as soon as it is emitted from a reaction, the ${}^2\text{He}$ nucleus breaks up into two protons, travelling in almost the same direction, and these can almost simultaneously activate two adjacent proton counters. The number of counts from two unrelated protons is very small.

They have used this detection system to study the $(\alpha, {}^2\text{He})$ reaction on ${}^{12}\text{C}$, ${}^{13}\text{C}$, and ${}^{16}\text{O}$ and find energy spectra very similar to those found for reactions with stable outgoing particles. It is therefore possible to use it to study the structure of the residual nuclei.

Some of their spectra are shown in the figure, and it is notable that very few states are excited, showing that the reaction is very selective. This means that the reaction will only go to states of a particular structure, and this enhances its value as a spectroscopic tool.

Examination of the states excited in the reactions mentioned shows that they are all of high spin. The final nuclei are ${}^{14}\text{C}$, ${}^{15}\text{C}$ and ${}^{16}\text{O}$ and the states excited are the 3^- at 6.73 MeV and the 4^+ at 10.55 MeV in ${}^{14}\text{C}$; the $5/2^+$ at 0.74 MeV in ${}^{13}\text{C}$; the $5/2^+$ at 0.74 MeV in ${}^{15}\text{C}$; and the 4^+ at 3.55 MeV in ${}^{16}\text{O}$.

The preferential excitation of high spin states has already been observed in

the (α, d) reaction and these states are favoured because the kinematics of the reaction are such that the neutron-proton pair is easily captured into a relative triplet state about an undisturbed target core. In the same way the observed selectivity in the $(\alpha, {}^2\text{He})$ reaction corresponds to the capture of a neutron-neutron pair into a relative singlet state. At 65 MeV, the energy of the alpha particles used in this experiment, the angular momentum transferred in a surface reaction is about four or five units of $\hbar$, so that states formed by capturing the two neutrons into d orbitals with the configuration $(d_{5/2})^2$ should be preferentially excited. The known spins of the states that are selectively excited are in agreement with this simple picture. Similar arguments can be used to predict the states that will be excited in reactions at different energies and on different nuclei.

These results already show the usefulness of the $(\alpha, {}^2\text{He})$ reaction in exciting states of high spin in nuclei. It is likely to be applied to study the structure of many nuclei throughout the periodic table. □

Pressure solution

from K. R. McClay

The Tectonic Studies Group of the Geological Society held a discussion meeting at Imperial College on the subject of pressure solution and Coble Creep, on November 5, 1976.

THE role of diffusive mass transfer processes in geological deformations, although indisputably important, has always been difficult to assess critically and even more difficult to quantify. Ever since Sorby, in the late 19th century, realised that some rocks exhibit textures and structures that can only be explained by 'solution' at highly stressed grain boundaries and redeposition of minerals in less stressed regions, structural geologists have been trying to understand this phenomenon of 'pressure solution'. This rock deformation mechanism has largely been ignored since the turn of the century, and only in the 1960s was interest in pressure solution revived, chiefly by J. Ramsay.

The meeting was intended to review current research on pressure solution and diffusion processes in rocks and also to evaluate their importance in geological deformations. Although the meeting was concerned with the fundamental aspects of pressure solution processes, these are also of considerable importance in the compaction behaviour of the reservoir rocks

in some oil fields.

Flow by diffusive mass transfer involves transport of matter from grain boundaries subjected to a high normal stress to grain boundaries which are less highly stressed. If the diffusion is predominantly through the grains, the process is termed Nabarro-Herring Creep, whereas if it is predominantly around grain boundaries, the flow is called Coble Creep. In general, grain boundary sliding must occur to accommodate the grain shape changes due to diffusion. These processes only proceed at a detectable rate, when the temperature is a large fraction of the absolute melting temperature of the material. Whereas processes involving solid state diffusion probably occur in high grade metamorphic rocks and in the mantle, geologists have long recognised that textures such as tectonic stylolites, truncated fossils and tectonic overgrowths in low grade metamorphic rocks often indicate deformation by diffusive mass transfer. Even over the relatively long time available for geological deformations, solid state diffusivities would be too slow to account for the observed strains. It is therefore inferred that the rate of diffusive mass transport is enhanced by the presence of an intergranular fluid film—hence the term pressure solution. Thus Coble Creep and pressure solution are geometrically similar in that they both involve intergranular diffusion.

E. Rutter (Imperial College) demonstrated that Coble Creep and pressure solution have similar flow laws (for a review see Rutter, *Phil. Trans. R. Soc. Lond.*, **A283**, 203; 1976), and indicated that the rate of diffusive mass transfer is generally considered to be the rate-controlling step during deformation. Rutter also emphasised the importance of recognising the several possible diffusion paths in low grade metamorphic rocks (R. Knipe, Imperial College). This research indicates that in some sandstones, intracrystalline diffusion along subgrain walls may be able to give rise to strain rates equivalent to those for pressure solution along grain boundaries. Stress relaxation experiments (E. Rutter and D. Mainprice, Imperial College), clearly showed the strong influence of pore water on the creep behaviour of Tennessee sandstone. They inferred that this behaviour indicates deformation by grain boundary sliding accommodated by diffusive mass transfer. In a review of the field evidence for pressure solution (such as striped cleavage, tectonic stylolites, and truncated fossils), J. Ramsay (University of Leeds) indicated that two types of pressure solution regimes occur; an isochemical regime where precipitation of the dissolved phase occurs close to the

generating interface (giving pressure shadows and overgrowths) and a regime where the bulk chemistry is changed because the dissolved material is precipitated elsewhere (giving tectonic veining). The influence of stress 'risers' which produce variations in the mean stress was considered important in the initiation of pressure solution seams.

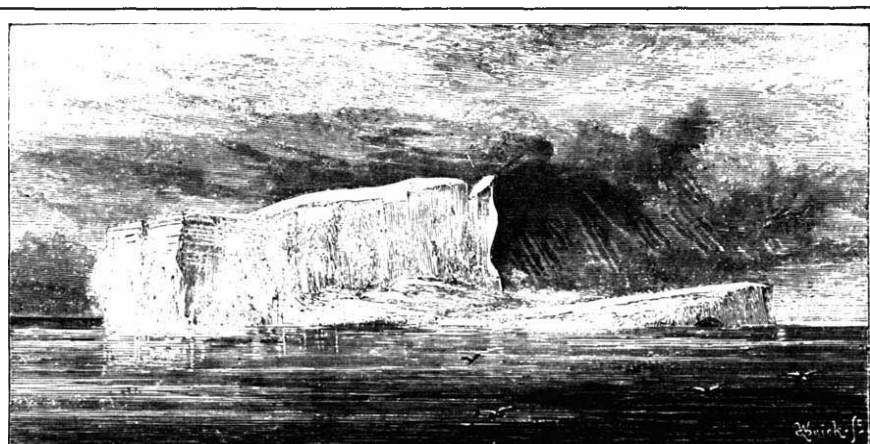
M. Casey (University of Leeds) has formulated a purely solid state, Coble Creep finite element model for diffusion over several grain diameters (that is, Ramsay's second regime). Applying this to a study of differentiation in microfolds and crenulations he has concluded that fluid-assisted diffusion is necessary to account for these microstructures. B. Burton (CEGB Laboratories, Berkeley) discussed the available data on Coble Creep in metals and ceramics, and emphasised that this may not be an important deformation mechanism at low stresses and low homologous temperatures because of the stress needed to create sources and sinks for vacancy diffusion.

R. De Boer (Shell Laboratories, Rijswijk) pointed out the difficulties in experimental investigations of pressure solution. The process is intrinsically slow and cannot be speeded up because of the low heat of activation, and also the difficulty in suppressing plastic deformation and cracking.

In experimental simulation of oil reservoir phenomena, he has found that pressure solution phenomena occur in quartz sands above 280 °C and in carbonate mixtures at 200 °C. An important report of recent research involving fluid inclusion geothermometry and oxygen isotope studies was

given by R. Kerrich (University of Western Ontario). He suggested that grain size greatly influences pressure solution processes and that these processes are important in the deformation of fine-grained quartz and carbonate rocks at low metamorphic temperatures. A lively discussion was generated by the contribution of A. Beach (University of Liverpool) who considered that many of the residue minerals in pressure solution seams are in fact the products of prograde metamorphism. Metamorphic reactions could produce the silica found in the tectonic veins common in low grade metamorphic environments.

Over the past decade much data has been collected and many significant advances made, and there has been a convergence towards detailed microstructural, isotopic and chemical studies of natural pressure solution systems. Although only limited success has been achieved so far in experimental work, stress relaxation tests will allow access to the slow strain rates which are not attainable in normal triaxial tests. Grain size effects are very important and careful consideration of the various possible diffusion paths must be made. Many questions still remain unanswered, however, particularly about the origin of the periodicity of pressure solution seams and striping. Many research programmes are being pursued however, and these will undoubtedly produce results which will not only be of great academic interest but also of significance in the interpretation of some fault behaviour, and compaction behaviour of oil field reservoir rocks. □



A hundred years ago

WE frequently saw table-topped icebergs with the upper surface very irregular; when that is the case evidence may usually be found from the colour, the closeness of the veining and other appearances that it is not the original surface of the iceberg which is now presented to us, but a second surface produced by cutting away by the sea of an entire story, as it were,

of the berg; which although it had no doubt at one time during the process been greatly inclined, had recovered its equilibrium on the whole of the upper layer having been more or less symmetrically removed. (Conditions in the Antarctic: from a lecture given by Sir Wyville Thomson in Glasgow.)

From *Nature*, **15**, December 7, 120; 1876.

review article

The Star of Bethlehem

David W. Hughes*

The Star of Bethlehem was probably a triple conjunction of Saturn and Jupiter in the constellation of Pisces, the significance of which was only obvious to the Magi of Babylonia. This occurred in 7 BC and events indicate that Jesus Christ was probably born in the Autumn of that year, around October, 7 BC.

THE Messianic star which heralded the birth of Jesus Christ and shone in the skies over Bethlehem, Judaea has been a phenomenon of considerable interest and much mystification for centuries. Almost every Christmas the star is pondered over and discussed. Invariably the same conclusions are drawn—the cause and form of the star are still uncertain. The astrologically significant triple conjunction of Saturn and Jupiter in Pisces is the most likely candidate but the two comets of March, 5 BC and April, 4 BC cannot be dismissed. Neither can the possibility that the star was simply legendary.

Biblical background

The principal account of the star is found in the gospel of St Matthew. It begins (Matthew II, 1–2):

“Now when Jesus was born in Bethlehem of Judaea in the days of Herod the King, behold, there came wise men from the east to Jerusalem, saying, where is he that is born King of the Jews? For we have seen his star in the east, and are come to worship him.”

The term “in the east” in the second verse is incorrect in this Authorised Version translation^{1,2}. Originally it was written *en té anatolé* (Greek singular) whereas “the east” is usually *anatolai* (Greek plural). *Anatolé* has a special astronomical significance, indicating the earliest visible rising of a star at day break (the helical rising), and so the second verse should read “for we have seen his star appear in the first rays of dawn”. These first two verses also indicate that Jesus was born and the visit of the wise men took place some time between 39 and 4 BC when King Herod was on the throne. Learning of the prophecy (Micah V, 2), “But thou, Bethlehem Ephratah, though thou be little among the thousands of Judah, yet out of thee shall he come forth unto me that is to be ruler in Israel.”, Herod told the wise men that this is where Christ would be.

“Then Herod, when he had privily called the wise men, enquired of them diligently what time the star appeared. And he sent them to Bethlehem and said, Go and search diligently for the young child; and when ye have found him bring me word again, that I may come and worship him also. When they had heard the king, they departed; and lo, the star, which they saw in the east went before them, till it came and stood over where the young child was. When they saw the star, they rejoiced with exceeding great joy. And when they were come into the house, they saw the young child with Mary his mother and fell down, and worshipped him: and when they opened their treasures they presented unto him gifts; gold and frankincense and myrrh.”

This second passage contains many interesting points. Very few people saw the star. Although the wise men saw it, Herod and

all Jerusalem did not, so the Christmas card image of a huge brilliant star illuminating all Bethlehem cannot be correct. It seems also that there were two separate appearances of the star. The first induced the wise men to leave the east and set out for Judaea; they then lost sight of it for a time. The second occurred when they were in Jerusalem and here it pointed out to them the place at Bethlehem where the object of their search was to be found. Not only did it point out Bethlehem, but it “went before them” and finally “stood over” Bethlehem. The star must have then been in the zenith. This ties in with the legend recounted by Maunder³. Apparently the star had been lost in the daylight by the time the wise men reached Jerusalem (indicating that it was an evening star during their journey). On reaching Bethlehem, apparently nearly at midday, one of them went to the well of the inn to draw water and, on looking down the well, saw the star reflected in the surface of the water—again indicating that it was in the zenith and that they had arrived at the right place. (For a discussion of seeing stars in wells during the day time, see refs 4 and 5.) It must be noted, however, that if a star is in the zenith it will appear to be equally over every object in the neighbourhood.

A star had been mentally associated with the coming of the Messiah for a long time. The oracle of Balaam (Numbers XXIV, 17) said,

“I see him, but not now;

I behold him, but not nigh:

a star shall come forth out of Jacob and a sceptre shall rise out of Israel.”

This idea is, in fact, attributed to a man whose home was near the river (Numbers XXII, 5), that is in Mesopotamia, where there was great interest in astrology and astronomical objects and where the Old Testament would be well known and substantively the same as it is now. Some present-day scholars⁶ however think the term “star” in Numbers (XXIV, 17) applies to the Messiah himself and not to the sidereal phenomenon heralding His appearance.

The third reference to the star is not in the Authorised Version of the *Bible* but in one of the infancy gospels that were omitted when the *Bible* was compiled. The Protoevangelium of James (XXI, 2) states⁷:

“And he (Herod) questioned the wise men and said to them:

‘What sign did you see concerning the new born King?’

And the wise men said: ‘We saw how an undescribably great star shone among these stars and dimmed them, so that they no longer shone, and so we knew that a King was born for Israel. And we have come to worship him’, and Herod said: ‘Go and seek and when you have found him, tell me, that I may also come to worship him’. And the wise men went forth. And behold, the star which they had seen in the east went before them, until they came to the cave. And it stood over the head of the child.”

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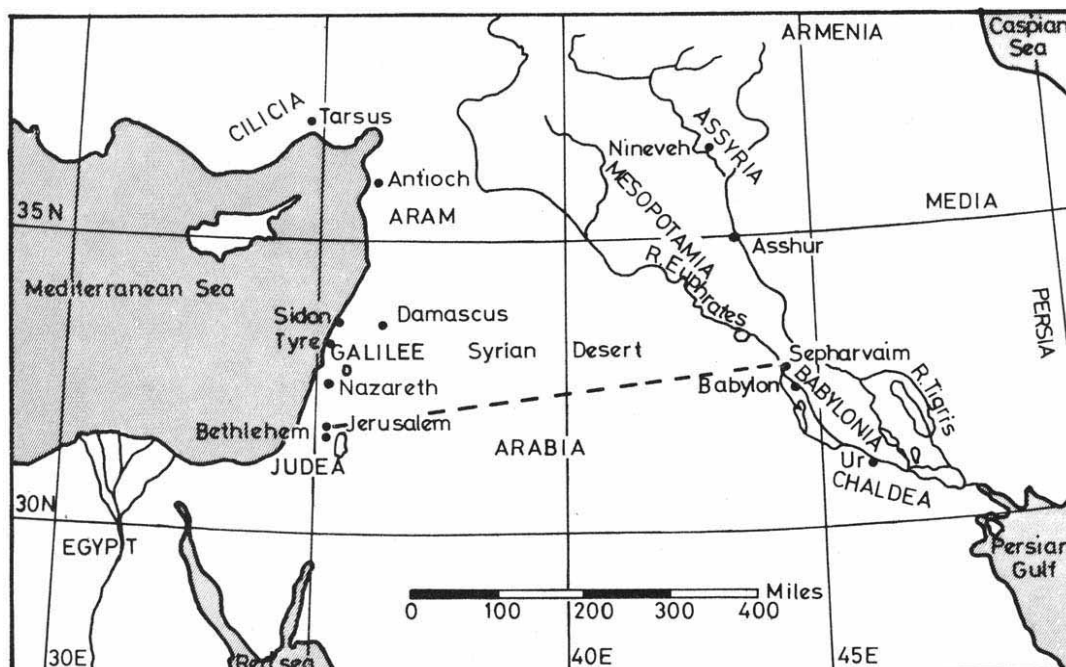


Fig. 1 The Middle East indicating one of the many possible routes of the Magi's journey from the east to Jerusalem and Bethlehem.

Here clues are given as to the star's brightness. To dim the other stars requires it to have a brightness comparable with or even brighter than the full Moon. But if this was so why didn't Herod and all Jerusalem know of it?

The wise men

The word Magi comes from the greek ($\mu\alpha\gamma\iota$) which the Authorised Version translated as "wise men" and the *New English Bible* as "astrologers". They were probably Median priests of Zoroastrianism who, apart from performing the duties of national priesthood, used to occupy themselves with the interpretation of dreams. Babylon, Assyria and Chaldea have been put forward as their place of origin (Fig. 1). It has also been suggested that Magi does not refer to a specific race at all but is just a general name for a priestly cast of "magical" tendencies. In the earliest carvings and pictures they are shown in Persian dress, wearing trousers. It is said that such a carving saved the Church of the Nativity of Ravenna from the general havoc of the Persian onslaught in AD 614. Astrology, the prediction of future events from the positional relationship between the Sun, Moon and planets and the stellar background, was definitely one of their practices.

In the language of the Old Testament and probably St Matthew as well the term "the east" was very vague and included countries that lay considerably to the north as well as the east of Palestine. Previous biblical books (Isaiah LX, 6; Psalms LXXII, 10, 15) predicted that the kings would come from Arabia (Sheba, Seba and Tarshish). But other biblical scholars have favoured Persia, Parthia (a country south-east of the Caspian that used to extend from the Indus to the Euphrates) and Babylonia. Wherever they came from they would have been expecting that a King of the Jews was to be born and have probably learnt of this from Jewish sources. (This expectation was widespread, compare Simeon in Luke II, 25.) The nature of the gifts does not present a clue.

It was not until the sixth century that tradition changed the Magi into kings. Their number was fixed at three because of the threefold nature of their gifts (eastern tradition has it that there were twelve).

The date

In AD 525 Dionysius Exiguus, a prominent scholar and Roman monk, fixed the origin of the present calendar so that Jesus was born in December, AD 1 (anno domini, "in the year of the Lord"). AD 1 corresponded to AUC 754 in the Varronian

reckoning (AUC standing for *ab urbe condita*, from the founding of Rome). Unfortunately Exiguus forgot the year zero which should have been inserted between 1 BC and AD 1 (in fact astronomical reckoning has AD 1, 1 BC 0, 2 BC

1 and so on) and also the 4 yr when Emperor Augustus ruled under his own name of Octavian. So Exiguus was at least 5 yr out.

Jesus was born in the days of King Herod. According to the historian Flavius Josephus⁸, Herod died within a few days of an eclipse of the Moon visible from Jericho and a few days preceding the feast of unleavened bread. The eclipse is the only one mentioned by Josephus⁹ and must have been that which occurred during the night of March 12-13, 4 BC. Passover started on April 11, so Herod died between March 12 and April 11, 4 BC. Christ must have been born before this. In fact when Herod died Christ was in Egypt with his parents, the family having taken refuge there after being warned of Herod's impending wrath.

On realising that the Magi had not returned to Jerusalem to tell him where the child was, but had gone back to their country another way, Herod sent men to kill all the children in Bethlehem and its environs who were 2 yr old or less "according to the time which he had diligently enquired after the wise men" (Matthew II, 16), so Christ could have been anything up to 2 yr old at this time.

Luke II provides another clue about the birth date. Joseph and Mary journeyed to Bethlehem from Nazareth because Caesar Augustus decreed that all the world should be taxed. An ancient inscription unearthed in Ankara¹⁰ lists the years in which orders were issued for tax collection. The most feasible date is 8 BC, but slow travel and communications in those days could have delayed the actual collection of taxes by a year or two. Luke also states that this taxing was first made when Cyrenius (Quirinius in Latin) was governor of Syria. Quirinius was a consul in 12 BC and also fought against the Homanadensians in Cilicia before 6 BC. Tertullian¹¹ states that the census at the time of the birth of Jesus was taken by Sentius Saturninus who was governor of Syria between 9 and 6 BC. Quirinius could have been associated with Saturninus in this project some time between 6 and 5 BC, when he was an Emperor's legate in Syria. But he did not become governor of Syria until AD 6 so Luke was mistaken (II, 2). Needless to say if Luke were correct in II, 2, Christ would have been born some time between AD 6 and 7 when Quirinius governed Syria.

Some early Christian writers (Origen and Eusebius, for

example) state that Jesus was 2 yr old when the wise men came; he was then taken to Egypt and remained in Egypt for 2 yr before returning to Nazareth. St Luke mentions neither star nor Magi in his gospel but tells of the first month or so of Christ's life, of His circumcision after 8 d and of His being brought to the temple in Jerusalem after Mary's purification.

All in all the above facts indicate that Jesus was born in 6 BC but some biblical scholars, with justification, plump for 7 BC and 5 BC. A brief chronology is shown in Fig. 2.

On which day of the year was Christ born? Christmas Day, December 25, is celebrated all over the world as the birthday of Jesus but this date has only been used since about AD 336 (ref. 12). In those times this date was accepted as the time of the winter solstice, midwinter's day, after which daylength increases. The pagan feast of *dies solis invicti natalis* (the birthday of the unconquered Sun) was celebrated on that day, which occurred near the middle of Saturnalia, a season during which work ceased, houses were decorated with laurels and evergreens, gifts were exchanged and parties and processions were held. As many early Christians would not give up this pagan holiday the western Church decided to transform the pagan ceremony into a Christian festival by having Christmas Day on December 25. In the east the birth was celebrated on January 6, a date to which also the star and the Magi, the baptism of Christ and the miracle at the wedding at Cana were attached. January 6 was also the date of the festival in the temple of Kore at Alexandria and at places in Arabia, celebrating Kore, the virgin, giving birth to Aion. Again the same date could have been chosen to replace a pagan ceremony by a Christian one. Epiphanius (AD 315-403) gives January 6, AUC 752 (2 BC) as the birthday.

Clement of Alexandria in the *Stromata* (AD 194) gives November 18, 3 BC but also states that others think the birthday was either May 20 or April 19/20. Epiphanius, 120 yr later, stated that May 20 (or May 21 or June 20) were the dates of the conception and not the birth so some confusion exists here. However the dates were fixed, they seem to agree with an old

tradition that Christ was conceived in the spring and born around midwinter. Keller² introduces another interesting complication. According to St Luke (II, 8), "there were in the same country shepherds abiding in the field, keeping watch over their flock by night". The climatic conditions in what was then Palestine during Christmas time are most inclement, there being an average 6-8 inches of rain during December and January. Bethlehem is also in the grip of frost during December, January and February and no sheep would be in the fields. Flocks are usually put out to grass between March and November, the shepherds being with the flocks during the lambing season in the spring (March and April).

The star

The star must have been a fairly long lasting phenomenon, to "go before" and "stand over", which rules out transient phenomena such as fireballs and very bright shooting stars.

What of the more lasting transients such as comets and novae (discounting the fact that a brilliant comet or nova would have been seen by Herod who, according to Matthew and James, had not seen anything)? Halley's comet was first seen in 240 BC and owing to its 76-yr periodicity, reappeared in 12 BC. It was first seen about August 25 near Mu Geminorum and moved by Beta Leonis, disappearing in Scorpio about 60 d later. It was seen all over China but no report of its observation in the west has come to light. This comet is No. 51 in Williams' catalogue¹³ and is too early to be the Star of Bethlehem. No. 54 (of AD 13) is too late. This leaves No. 52 which appeared in March, 5 BC in the constellation of Capricorn and lasted 70 d, and No. 53 which appeared in April, 4 BC in Aquila and was possibly a nova being referred to in the Chinese as a "comet without a tail".

Some people think that Venus was "the star" and at the latitude of Bethlehem Venus is high enough in the sky to be a most impressive sight, being at times 15 times brighter than Sirius, the brightest star. But it is most unlikely that the Magi,

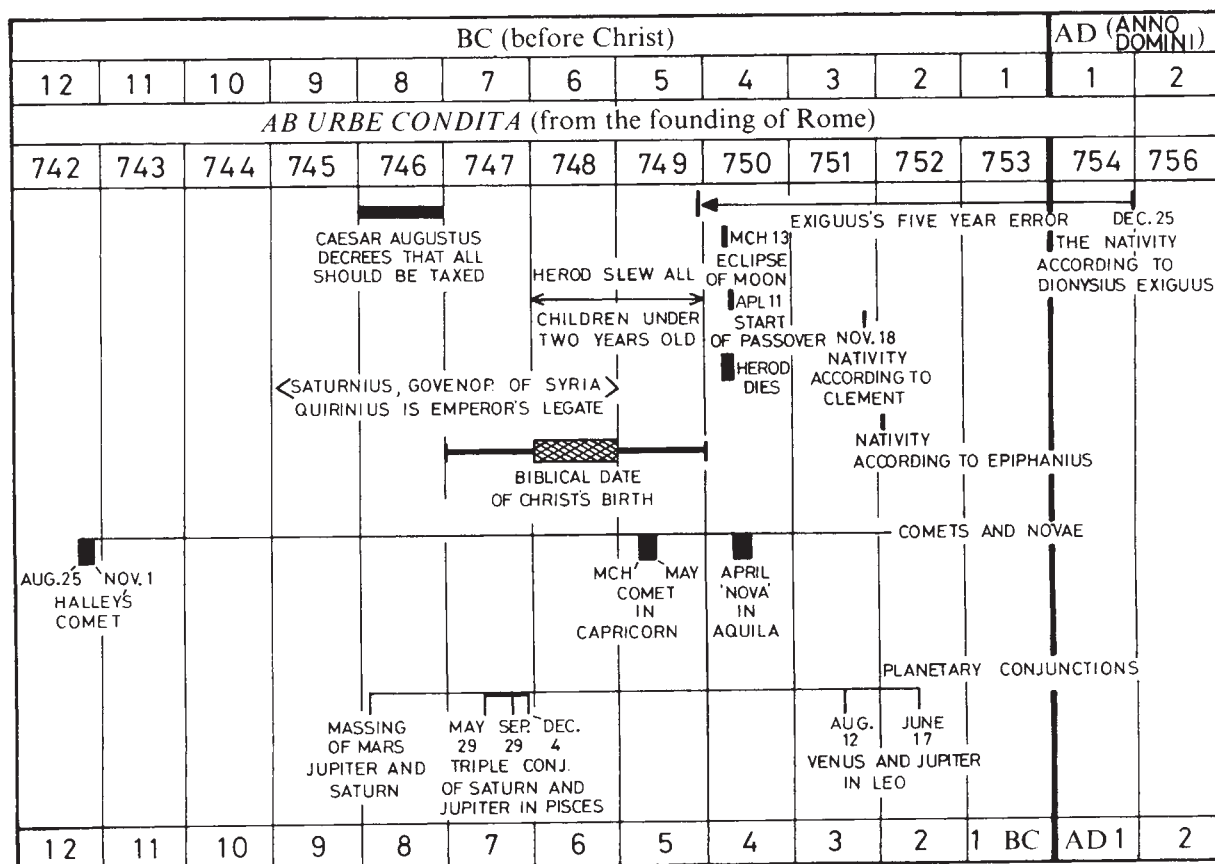


Fig. 2 A chronology of the major biblical and astronomical events occurring around the time of the birth of Christ.

constant observers of the sky, would be caught unawares by an appearance of Venus.

Another possibility is that the star was simply the conjunction between two or three planets. Two or more bright planets very close together in the sky provide a most striking sight. Johannes Kepler was fascinated by the conjunction between Jupiter and Saturn on December 17, AD 1603. In the spring of 1604 Mars moved into the vicinity of the other two and in autumn 1604 a supernova (SN 1604 Ophiuchi) appeared nearby. All in all, Jupiter and Saturn came into conjunction three times in 6 months. Jupiter and Saturn have orbital periodicities of 12 and 29 yr, respectively, so Jupiter on average passes Saturn every 20 yr. About every 120 yr, three successive conjunctions take place over about 6 months, this being known as a triple or great conjunction. Such a conjunction will occur every 120 yr and a similar one would have occurred in 7 BC. Pritchard^{14,15} confirmed this fact, and on May 29, September 29 and December 4, 7 BC a conjunction occurred in the constellation of Pisces, which is astrologically associated with the Jewish people. In February, 6 BC Mars moved into the configuration and formed an equilateral triangle with the other two planets, a situation known as a massing of planets¹⁶, but the Sun was too close for this to be observed with the naked eye.

Pritchard¹⁵ points out that in 66 BC, about two revolutions of Saturn and five of Jupiter before 7 BC, a closer single conjunction of the two planets took place, again in Pisces. In February, 66 BC the planets would set about 45 min after the Sun and would be less than 55 min of arc apart. Whether the astrological significance to the Magi of this single conjunction was the same as the significance of a triple conjunction is unknown. Maybe these previous conjunctions also impelled Magi to journey to Jerusalem, but the Bible only reported the fruitful journey.

Sinnott¹⁷ studied the 200 conjunctions and 20 multiple groupings that occurred between 12 BC and AD 70, these being gleaned from Tuckermann's book¹⁸. Sinnott concentrated on very close groupings, "weeding out" commonplace conjunctions and ones which were widely separated, and lists six conjunctions which could be seen from the Middle East in which the stars were less than 12 min of arc apart and more than 15° from the Sun (so they easily could be seen at night). These are shown in Fig. 2. Unfortunately none occurred between 12 BC and 3 BC, indicating (if the above thoughts about when Christ was born are correct) that Sinnott's selection criteria are too strict. The two conjunctions of the brightest planets, Venus and Jupiter, the first on August 12, 3 BC, visible in the eastern dawn sky from about 0345 h to 0520 h when they were separated by about 12 min of arc (in Leo, near Regulus) and a similar event on June 17, 2 BC when they were less than 4 min of arc apart and close to the western horizon where they would seem to fuse into one star "gleaming like a great beacon over Judaea to the west", would have been most impressive but do occur after Herod's death. The second conjunction again occurred near Regulus, a royal star. (Astrologically it is interesting that Judah, one of the tribes of Israel, has the lion, symbolising the constellation Leo, on its standard. Bethlehem, in the land of Judah, was the predicted birthplace of a "Governor that shall rule my people Israel" (Matthew II, 6). These facts were well known to the Magi.)

Possible sequences of events

It must be remembered that the Magi were skilled astrologers and accomplished star gazers with great knowledge of the sky, backed up by about 4,000 yr of astronomical observations. Many towns in the Middle East have legends retelling how theirs was the town from which the Magi started on their journey (the people of Saveh in Persia told Marco Polo that their town was the starting point). The triple conjunction of 7 BC had been predicted. The calculations and predictions are given in an almanac found on a cuneiform tablet at Sippar (Sefarvaim) in Babylonia, a town noted for its school of

astrology. The conjunction took place in Pisces, a constellation which is astrologically associated with the Jewish people and Israel (compare the writings of Rabbi Arbarband 1437–1508). The Sun moves through Pisces at the boundary between winter and spring and astrologically this indicates the end of an old age and the start of a new one. Jupiter was considered to be a lucky and royal star and in traditional Jewish astrology Saturn protected Israel.

Babylonian astrologers steeped in Jewish tradition would have predicted the triple conjunction in Pisces. Realising its astrological significance (that is, a Jewish king born in Israel) and remembering the prophecy of Balaam (Numbers XXIV, 17) and seeing in the sky their astronomical prediction come true would be ample justification for starting a journey to pay homage to the new king, the herald of a new age.

The journey from Sefarvaim to Jerusalem across the Arabian desert is about 550 miles. Preparing for and taking this journey would take about 4 months. The planetary clustering would have begun about the end of February, 7 BC with Jupiter moving from Aquarius towards Saturn in Pisces. As the Sun was also in Pisces at this time the planets would not be visible. Not until April 12 did both planets rise helically in Pisces (compare Matthew II, 2). The first close encounter (0.98 degrees separation—twice the diameter of the full Moon) took place on May 29 and was visible for about 2 h in the morning sky. The Magi could have set out towards the end of June, 7 BC, the September 29 conjunction confirming their predictions as they were nearing Jerusalem and the December 4 conjunction, occurring after the audience with Herod, pointing the way south to Bethlehem 5 miles away. Pisces rises at about 0200 h, 2200 h, 1900 h and 1200 h during May, July, September and December, indicating that the two planets would be clearly visible during their journey. The planets are due south (and closest to the zenith) about midnight during September and at about 1900 h during early December. (This throws doubt on to the Maunder legend of the midday star in the well.) Jesus could have been born about 2 months before the last conjunction, say in early October, 7 BC. He would have been circumcised at 8 d, then after the 33 d of Mary's purification taken to Jerusalem where a sacrifice was made. After returning to Bethlehem he was visited by the Magi. (The word *τοῦτο* in Matthew II, 11 means "a very young child" or infant.) After being warned in a dream about Herod's impending wrath, Joseph fled with Mary and Jesus to Egypt. In early October the shepherds could be moving the sheep from the hills to their winter quarters. Jesus would have been 2.5 yr old when Herod died.

A second scenario could be as follows. The conjunctions of 7 BC could simply have attracted the wise men's attention towards Palestine, the comet of March, 5 BC starting them on their journey. They would have arrived well before Herod's death (which occurred between March 12 and April 11, 4 BC) and being told that Jesus was born in Bethlehem, arrived there about April, 4 BC when the nova was shining. Before Herod died he ordered that all children under 2 yr old be killed, and with this scenario the order would have gone out just before he died, giving Joseph, Mary and Jesus time to flee to Egypt.

Jesus would have been about 2 yr old when this order went out and when seen by the wise men. This ties in with Epiphanius⁸ who stated that the star shone in the east 2 yr before the Magi arrived in Jerusalem. So Jesus would have been born in the spring of 6 BC, Epiphanius's star could have been the conjunction and the shepherds would have been in the fields at lambing time.

But why should Joseph and Mary stay in Bethlehem for 2 yr when they had only gone there to be taxed? And why had not Herod and the Jews connected the comet of 5 BC with the coming of Christ? It is possible that Herod missed the triple conjunction when the planets only just came within 1° of each other. The Protoevangelium of James supports this latter picture.

Non-astronomical possibilities

One easy way out of the dilemma introduced by the Star of Bethlehem is to regard it as another miracle, as the direct intervention of the hand of God and thus as a phenomenon not requiring a scientific explanation.

It could also just be a legend. No king worth his salt in those days was born without some celestial manifestation. A star greeted the birth of Mithridates (131–63 BC) and Alexander Severus¹⁰. The temple of Diana at Ephesus was burned down the night that Alexander the Great was born, Magi announcing that the plague and bane of Asia had been born that night. The magos Tiridates and some followers came to Rome to acknowledge the divinity of Nero in AD 66.

Matthew, who wrote his gospel for a Jewish audience and who frequently cited passages of the Old Testament to prove points to his readers, probably felt that a star was necessary to fulfil the prophecy of Balaam. The Essenes, who studied the prophets to find interpretations of the contemporary scene, would believe that such a prophecy could not have been without fulfilment. They would go so far as to say: no star, no messiah. The absence of the Matthean clause "that it might be fulfilled" is very worrying and casts doubts as to how far the evangelist regarded the account of the star as historical. Albright and

Mann¹ suggest that the clause might have been "edited out" of Matthew's gospel at a later date to appease Gnostic opposition to the church. (The gospel of St Matthew was probably written some time between AD 65 and AD 85.)

Bearing in mind Herod's surprise on being told of the star by the Magi, the triple conjunction in 7 BC seems to be the most probable candidate. If this is so Christ would have been born in about October of that year.

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articles

Nuclease cleavage of chromatin at 100-nucleotide pair intervals

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Digestion of mouse liver nuclei with DNase II leads to a novel cleavage pattern with a 100-nucleotide pair periodicity. From chromatin, this pattern or the standard 200-nucleotide pair repeat can be produced depending on the ionic conditions. The results are interpreted by assuming different conformational states of the nuclear material, including condensed and extended forms.

THE discovery of a chromatin subunit has had a great impact on the concept of the structure of chromatin (for reviews see refs 1–3). Evidence for this subunit, which has also been termed ν -body or nucleosome rests mainly on results obtained from nuclease digestion of nuclei and on direct visualisation of particulate structures in the electron microscope. Approximately 200 nucleotide pairs of DNA seem to be wrapped around a globular histone bead which has been proposed to consist of two of each of the histones H2A, H2B, H3 and H4. Digestion of nuclei with an endogenous nuclease or micrococcal nuclease yields protected DNA fragments of 200 nucleotide pairs and multiples thereof, presumably by attack in a region between the subunits. Within the nucleosome, cleavage at preferred sites has been reported for micrococcal nuclease⁴ and for DNase I which introduces single-strand nicks at intervals of ten nucleotides into the nucleosomal DNA⁵.

We have studied various aspects of chromatin structure with the help of restriction nucleases⁶ and by a detailed investigation of the action of micrococcal nuclease^{7,8}. It seemed promising to extend the work to DNase II which has already been used in a few laboratories to digest chromatin^{9–11}. We have used this nuclease for several reasons: it introduces into DNA single-strand as well as double-strand breaks¹², it has no requirement for divalent cations in contrast to all other nucleases used for the digestion of chromatin, and even though its pH optimum is around 5, it retains sufficient activity at neutral pH to permit its use in physiological conditions¹². One unexpected outcome of our experiments was the finding that DNase II can cleave the DNA in chromatin and nuclei with a previously unobserved 100-nucleotide pair periodicity.

100-Nucleotide pair repeat pattern from mouse nuclei

Mouse liver nuclei were treated with DNase II and the DNA was analysed by Agarose gel electrophoresis. The resulting pattern is shown in Fig. 1, together with the pattern found with micrococcal nuclease in the same conditions. The positions of the peak maxima in the DNase II pattern correspond to sizes which are multiples of 100 nucleotide pairs (Fig. 1B), that is half the value obtained with micrococcal nuclease. The gradual shortening

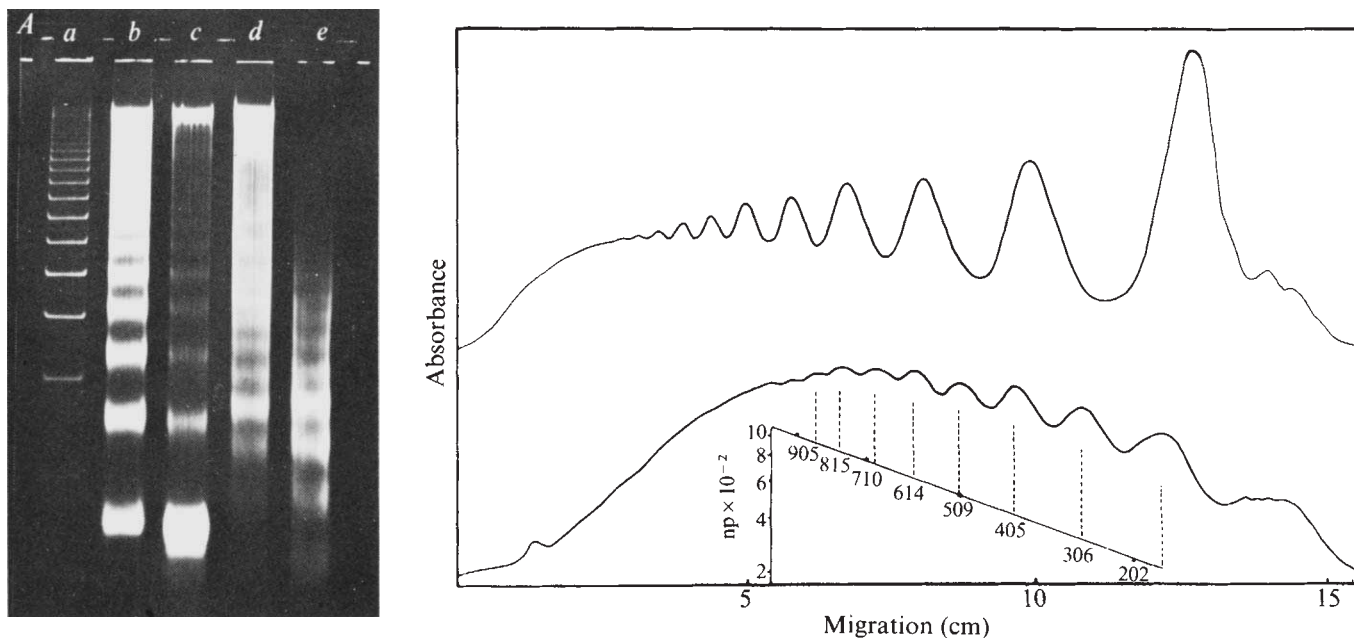


Fig. 1 Digestion of mouse liver nuclei with DNase II and micrococcal nuclease. Mouse liver nuclei were prepared as described before⁶. Samples containing 100 μ g of DNA were suspended in 10 mM Tris-HCl, pH 7.0, 1 mM phenylmethane sulphonyl fluoride (PMSF) in a total volume of 0.2 ml. Microscopic inspection showed the nuclei to be largely intact in these conditions. *A*, Incubations were for 1 h at 37 °C. In (*b*) and (*c*) micrococcal nuclease (Worthington) was present at concentrations of 7 and 25 U ml⁻¹, respectively, and the incubation buffer was supplemented with 1 mM CaCl₂. In (*d*) and (*e*) DNase II (Worthington) was added to concentrations of 125 and 250 U ml⁻¹, respectively. Slot (*a*) contains a mouse satellite DNA marker partially digested with *Eco*RII¹³. After incubation, DNA was extracted as described before⁶ and 5 μ g was applied to a 1.8% vertical Agarose slab gel, run in the Tris-borate buffer system as described before¹⁴. It was afterwards stained with ethidium bromide (1 μ g ml⁻¹) for 30 min. *B*, DNA from nuclei incubated with DNase II (250 U ml⁻¹) as in (*A*) was electrophoresed in a 1.8% Agarose gel with and without admixed mouse satellite DNA, partially digested with *Eco*RII. The gel was stained with ethidium bromide and photographed, and the negative was scanned as described before⁶. The lower tracing shows the DNase II pattern: in the upper tracing DNA from a micrococcal digest of nuclei is shown for comparison. Inset, average sizes of the DNase II fragments are depicted as they are determined in the coelectrophoresis with the satellite DNA digest. The molecular weights of the satellite fragments had been determined (R. E. Streeck, to be published) relative to the size of the sequenced restriction nuclease fragment H of SV40 virus (ref. 15 and W. Fiers, personal communication).

of the DNA fragments during digestion with micrococcal nuclease was not observed with DNase II. This may explain why the fragments in the DNase II digests seem to be slightly larger than the corresponding fragments from micrococcal nuclease digests (Fig. 1). A kinetic analysis of DNase II digestion (Fig. 2) indicates that the 100-nucleotide pair repeat is present as soon as discrete bands can be resolved, and persists throughout digestion.

To rule out the possibility that the 100-nucleotide pair periodicity might appear only in rather special conditions of digestion and possibly be due to a minor activity present in the commercial enzyme preparation used, we tested a wide range of incubation conditions. The intensity of the bands relative to the background turned out to be highest in the low ionic strength conditions used. The bands were also detectable, however, when nuclei were digested in the presence of up to 5 mM EDTA or 5 mM CaCl₂ or up to 150 mM NaCl. Concentrations of CaCl₂ or EDTA in excess of 10 mM prevented degradation altogether. Variation of the pH between 4.0 and 7.5, and a change to phosphate and acetate buffers, did not abolish the pattern. It was found reproducibly with several batches of DNase II as well as with a preparation of DNase II prepared in the laboratory of G. Bernardi¹². The pattern is also not an exclusive property of mouse nuclei since it was found with rat liver^{7,8} as well as calf thymus nuclei (M. Steinmetz, R. E. Streeck and H.G.Z., manuscript in preparation).

It is impossible to differentiate quantitatively between DNA falling into the 100-nucleotide pair repeat pattern and randomly cleaved DNA which contributes to the background in the gels. Therefore we cannot rule out the possibility that only a certain part of the nuclear material is cleaved with a 100-nucleotide pair periodicity in these experiments.

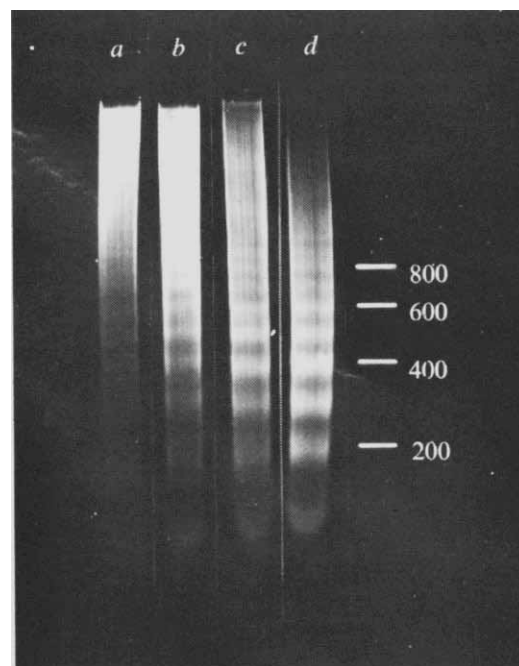


Fig. 2 Kinetics of digestion of mouse liver nuclei with DNase II. Nuclei were digested with DNase II (120 U ml⁻¹) as described in Fig. 1. Samples containing 5 μ g of DNA were withdrawn after 5 (*a*), 8 (*b*), 11 (*c*) and 18 (*d*) min, and analysed on a 1.8% Agarose gel. The molecular weight scale (in nucleotide pairs) was derived from *Eco*RII-digested mouse satellite DNA (Fig. 1).

Protease activity, as one potential source of artefact, was monitored during DNase II action by analysis of the histone patterns. In the presence of PMSF, which was always used, no differences were observed between unincubated samples and samples incubated without enzyme, with DNase II, and with micrococcal nuclease for the incubation periods used.

DNase II resembles DNase I in its ability to introduce single-strand nicks at 10-nucleotide intervals into the DNA. In denaturing 6% polyacrylamide gels¹⁶, DNA from DNase II-digested nuclei gave a series of strong bands with a 10-nucleotide repeat up to a fragment of 180 nucleotides, similar to the pattern found with DNase I¹. In addition, broader bands were present at 200, 300, 400, 500 and 600 nucleotides.

New chromatin subunit?

Nucleosomes can be isolated as deoxyribonucleoprotein particles by sucrose gradient centrifugation. It was conceivable, therefore, that by this technique discrete deoxyribonucleoprotein particles containing 100-, 200-, 300-, and so on, nucleotide pair DNA, could be observed after digestion with DNase II. Figure 3 shows that this is not the case in the conditions used, but that instead particles are seen which correspond in their sedimentation properties to those produced with micrococcal nuclease.

Quantitatively, however, the patterns do differ. In the DNase II digests, there is always, even after extensive digestion, little material in the monomer region and the peaks with even numbers are more pronounced than the others. If the DNA from the gradient fractions is analysed in Agarose gels (Fig. 3B) it is evident that this DNA is of the 100-nucleotide pair repeat type, but the rapidly sedimenting material contains not just the high multiples of the repeat unit but the entire spectrum of bands starting with the 100-nucleotide pair fragment. We interpret this to mean that one site of attack for DNase II is between nucleosomes, and that cleavage at the second site, within the nucleosome, does not lead to the appearance of a smaller particle, but that instead the integrity of the subunit is maintained. In addition, the small amount of monomeric nucleosomes and the high proportion of dimers and tetramers in the sucrose gradients indicate that these nucleosomes have a high tendency to remain associated even when the internucleosomal DNA is broken.

Histone H1 is implicated in internucleosomal interaction and might be responsible for aggregation between nucleosomes. To test this, H1 was dissociated by 0.6 M NaCl after DNase II digestion. This changed the sucrose gradient profile drastically (Fig. 4A). Much more monomer was produced at the expense of higher multiples, and the DNA analyses of the gradient fractions indicate that the aggre-

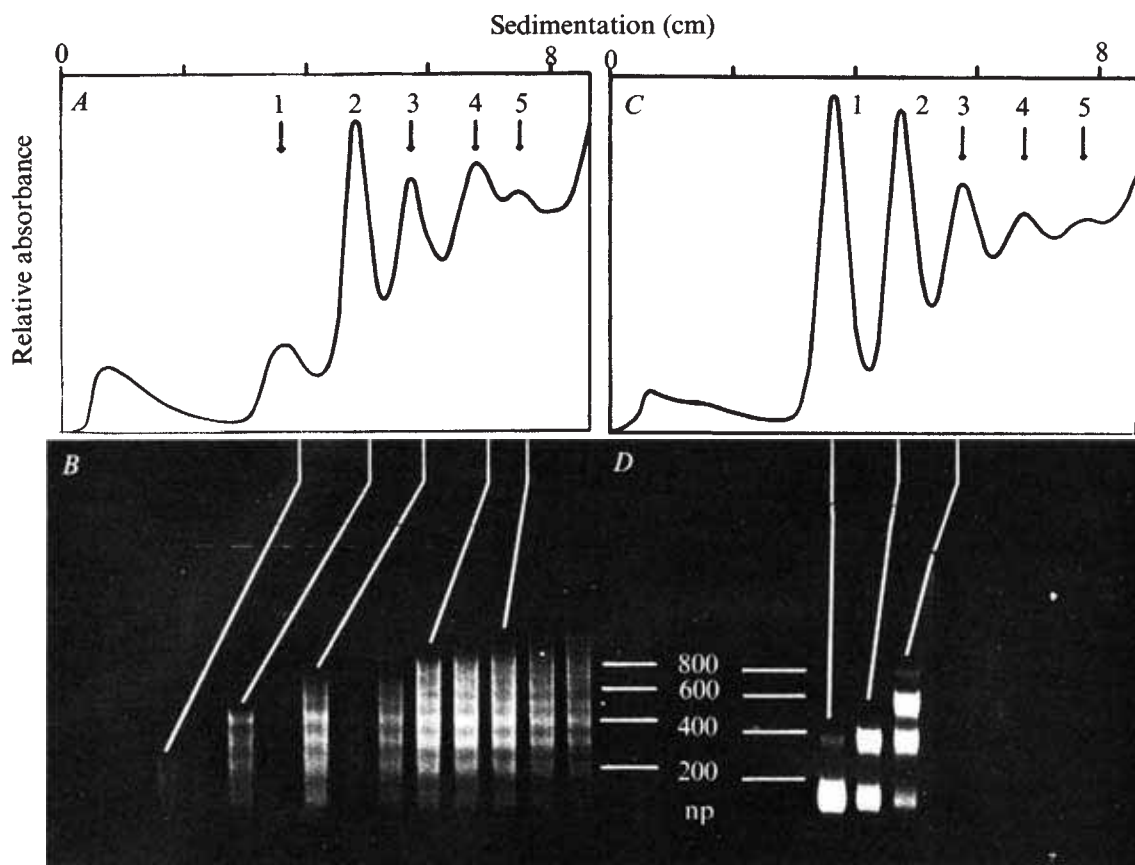


Fig. 3 Sucrose gradient fractionation of nuclease digests of mouse liver nuclei. Nuclei were isolated as in Fig. 1, except that 0.2% Triton X-100 and 1 mM PMSF were included in the homogenisation medium. They were digested with DNase II (120 U ml^{-1}) for 60 min at 37°C in 10 mM Tris-HCl, pH 7.0, 1 mM PMSF or with micrococcal nuclease (10 U ml^{-1}) in identical conditions except that 1 mM CaCl_2 was present during incubation. At the end of the incubation 2 mM EDTA was added, and the mixtures were centrifuged at low speed ($4,000g$, 10 min). The pellet was resuspended in 0.2 mM EDTA, pH 7.0, insoluble material was removed by a low speed spin and the supernatant was applied to isokinetic sucrose gradients containing 1 mM EDTA, pH 7.0 ($C \approx 5\%$, particle density 1.51, ref. 17). This supernatant contained negligible residual DNase II activity as shown by control incubations of the supernatant at 4°C for 24 h. Centrifugation was in a Spinco rotor SW 41 for 13 h at 38,000 r.p.m. at 4°C . The gradients were fractionated from bottom to top as described¹⁷ and absorbance at 260 nm was monitored in a flow-through cell with a Zeiss spectrophotometer. A, DNase II digest; C, micrococcal DNase digest. The numbers refer to monomeric (1), dimeric (2) and so on nucleosomes. DNA was isolated from the nucleosome fractions of both gradients and analysed on 1.8% Agarose gels (B and D) as described in Fig. 1. The solid lines correlate the nucleosome peak fractions with the corresponding DNA samples. The molecular weight scale (in nucleotide pairs) was derived from *Eco*RII-digested mouse satellite DNA (Fig. 1).

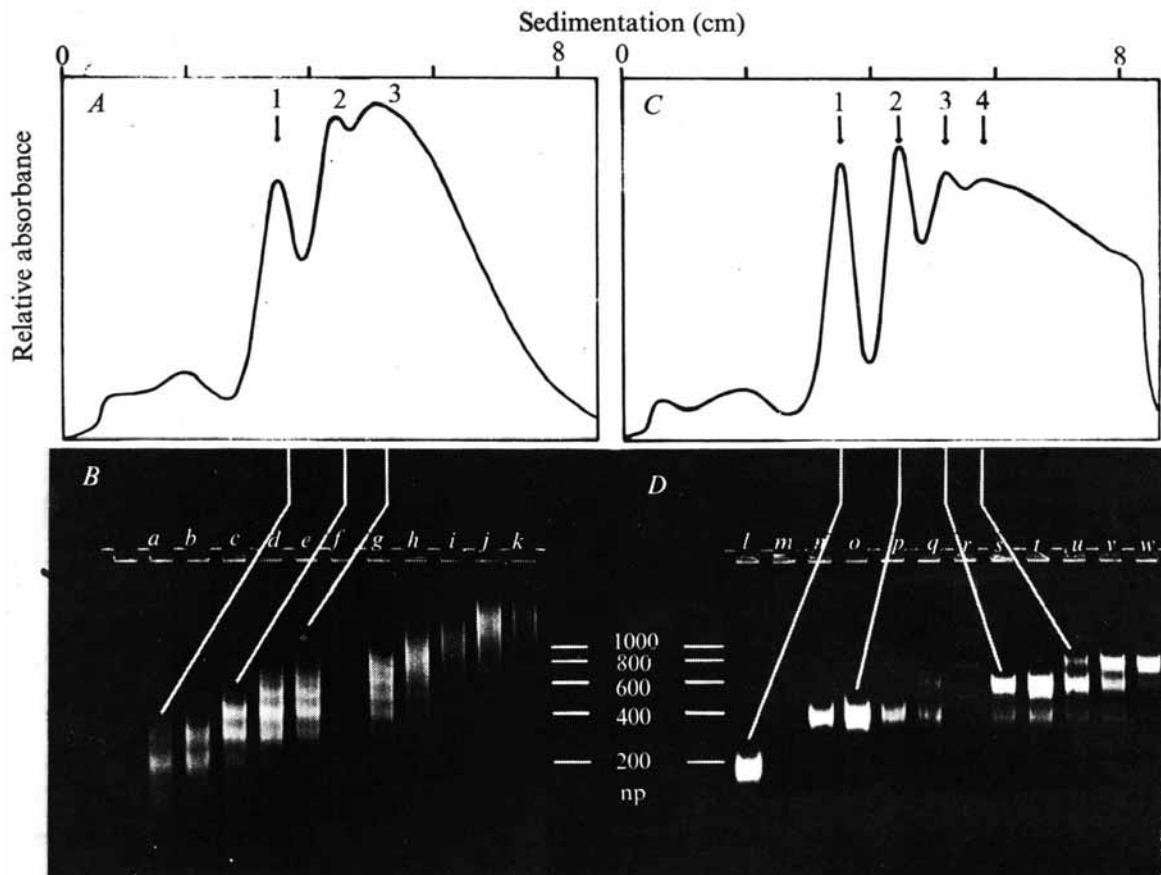


Fig. 4 Sucrose gradient analyses of nuclease digests after exposure to 0.6 M NaCl. The same nucleosome-containing supernatants as those from Fig. 3 were made 0.6 M in NaCl and then analysed as described in Fig. 3 without inclusion of NaCl in the gradients. *A* and *B*, DNase II; *C* and *D*, micrococcal nuclease digests. Slots (*f*) and (*r*) contain *Eco*RII-digested mouse satellite DNA.

gation between nucleosomes was strongly reduced (Fig. 4*B*). Nevertheless, the integrity of the nucleosome was maintained in spite of the internal cut. There is also aggregation between nucleosomes in micrococcal nuclease digests which

is abolished by exposure to 0.6 M NaCl. This is not so apparent from the sucrose gradient profiles (Figs 3*C* and 4*C*), but clearly seen in the gel analyses of sucrose gradient fractions (Figs 3*D* and 4*D*). In both micrococcal nuclease and DNase II digests nucleosomes sediment more slowly after exposure to 0.6 M NaCl, indicative of the loss of histone H1 and possibly a conformational change in the nucleosomal arrangement produced by the salt step.

DNase II digestion patterns sensitive to ionic environment of chromatin

For some experiments chromatin was prepared from the nuclei as a first step towards a more defined system. Mouse liver nuclei were suspended several times in 20 mM EDTA, 80 mM NaCl, pH 6.3 (ref. 18), and then in the usual 10 mM Tris-HCl (pH 7.0) and 1 mM PMSF. The unshredded gel-like material gave the typical pattern after digestion with micrococcal nuclease (Fig. 5*b*). The pattern obtained with DNase II from this chromatin depended very much on the ionic conditions. In the standard buffer, the DNA was cleaved by DNase II into a 200-nucleotide pair repeat pattern (Fig. 5*c*) similar to the micrococcal nuclease pattern. The 100-nucleotide pair repeat pattern

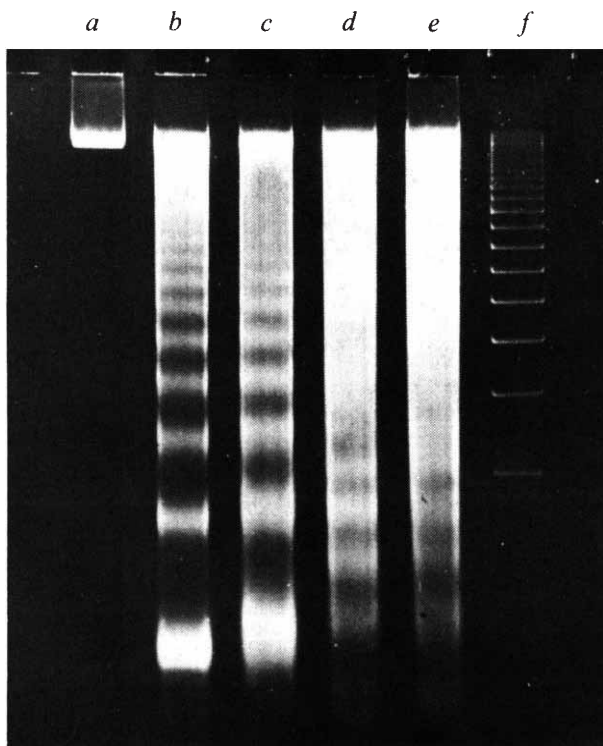


Fig. 5 Digestion of mouse liver chromatin with DNase II and micrococcal nuclease. Chromatin was prepared by extracting mouse liver nuclei three times with 80 mM NaCl, 20 mM EDTA, pH 6.3 (ref. 18). The resulting chromatin was washed twice in 10 mM Tris-HCl, pH 7.0 and digested in the same buffer in the presence of 1 mM PMSF for 40 min at 37 °C. Additions were: *a*, 1 mM CaCl_2 ; *b*, 1 mM CaCl_2 , micrococcal nuclease (25 U ml^{-1}); *c*, DNase II (500 U ml^{-1}); *d*, 1 mM CaCl_2 , DNase II (500 U ml^{-1}); *e*, 150 mM NaCl, DNase II (500 U ml^{-1}). Slot (*f*) contains an *Eco*RII digest of mouse satellite DNA (Fig. 1).

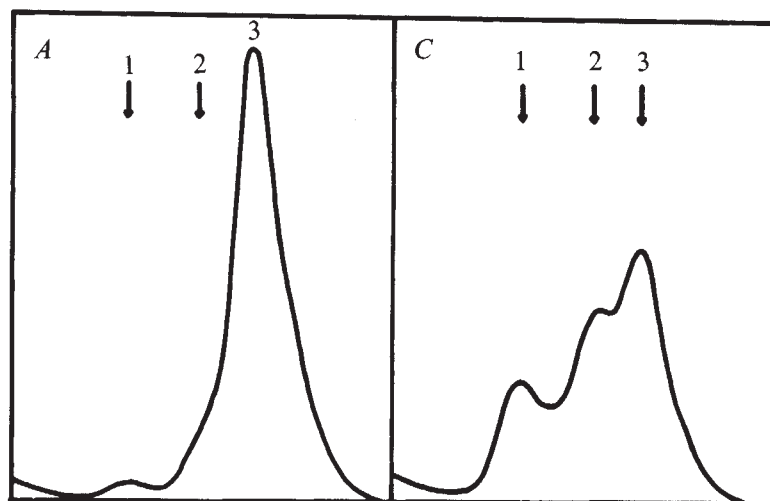
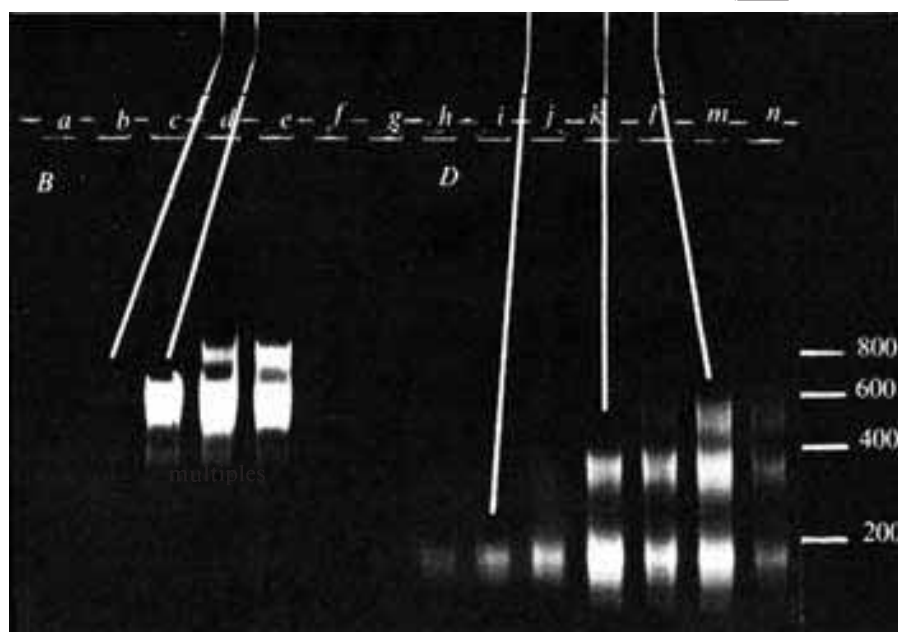


Fig. 6 DNase II digestion of a trimeric nucleosome fraction from a micrococcal digest. The micrococcal digest of nuclei was prepared and fractionated on sucrose gradients as described in Fig. 3. The material corresponding to trimeric nucleosomes was collected, concentrated and adjusted to 0.2 mM EDTA, pH 7.0, by pressure dialysis (Amicon), recentrifuged in a sucrose gradient as before and concentrated again by pressure dialysis. It was then incubated in 10 mM Tris-HCl, pH 7.0, 0.2 mM EDTA and 1 mM PMSF for 5 min without (A and B) and with DNase II (90 U ml⁻¹) (C and D). The incubation mixtures were analysed in sucrose gradients containing 1 mM EDTA, pH 7.0, in a Spinco rotor SW 56 for 14 h at 32,000 r.p.m. at 4 °C. In (A) and (C) the gradient profiles are shown, and in (B) and (D) analyses of the DNA from the gradient fractions as correlated by the solid lines.



appeared, however, if DNase II digestion was carried out after prior addition of divalent or monovalent cations (Fig. 5d and e). This shift from a 200-nucleotide pair to a 100-nucleotide pair pattern coincides with strong contraction of chromatin known to be caused by these salt conditions¹⁹.

200-Nucleotide pair repeat pattern with DNase II

Cleavage at 200-nucleotide pair intervals by DNase II at low salt conditions might take place at either the inter- or the intranucleosomal sites. To decide between the two possibilities, sucrose gradient analyses and codigestion experiments were carried out with micrococcal nuclease.

Sucrose gradients of DNase II digests, which on deproteinisation yielded the 200-nucleotide pair repeat pattern, gave the standard nucleosome pattern (not shown), as would have been expected only if cleavage had occurred between nucleosomes. To define this region further relative to the region where micrococcal nuclease cleaves, trimeric nucleosomes were isolated by sucrose gradient centrifugation from a micrococcal nuclease digest of mouse liver nuclei and further digested with DNase II. In standard conditions, without any salt added, dimeric and monomeric nucleosomes were produced, containing only DNA fragments of the 200-nucleotide pair repeat length (Fig. 6). This proves that the regions cleaved by micrococcal nuclease and by DNase II in these conditions must overlap either completely or at least partially.

To find out whether DNase II digestion of trimeric nucleosomes also depends on the ionic conditions, digestion was carried out in the presence of CaCl₂ or MgCl₂ at concentrations between 0.5 and 2 mM (ref. 8). The patterns were clearly different when divalent cations were present: there were DNA bands in the gel at 200 and 400 nucleotide pairs and there was also DNA migrating in between these bands with indications of bands around 300 and 500 nucleotide pairs. The patterns were not sufficiently defined, however, to identify them clearly as of the 100-nucleotide pair repeat type.

Analogous results were obtained with two other nucleosome fractions, one containing tetramers, the other pentamers to dodecamers. Clear 200-nucleotide pair repeat patterns were seen after digestion with DNase II in standard conditions and a high background and indications of intermediate bands when divalent cations were present in the incubations.

DNase II and structural features of the nucleosome

The major difference between DNase II and other nucleases is the preferential double-strand cleavage at one site within the nucleosome. This difference might be due in part to the ability of DNase II to introduce both double-strand and single-strand cuts with high frequency¹².

It is conceivable that double-strand cleavage in the nucleosome occurs more easily after several primary single-strand cuts. At one site, cleavage would then occur in both strands. The stronger propensity of DNase II to nick DNA intranucleosomally might also be responsible for the high background of DNA in the digestion patterns. This interpretation is supported by the finding in our laboratory that DNase I, a nuclease which introduces predominantly single-strand cuts into DNA, also produces a 100-nucleotide pair repeat pattern, albeit much weaker and with an even higher background of DNA between bands*. That DNase I has a preferred site of attack within the nucleosome has been proposed on the basis of a strong subnucleosomal band in denaturing polyacrylamide gels²⁰.

What features of the chromatin structure lead to DNase II cleavage at the intranucleosomal site? Splitting at this site is symmetrical as far as the DNA is concerned, and in view of the symmetrical construction of the nucleosome from two of each of the core histones, it is tempting to think that DNase II might split at a site of symmetry within the nucleosome. Several models for the nucleosome structure incorporate such a symmetry element (for example refs 21 and 22). Extending the symmetry to include histone H1 would require two molecules of H1 per nucleosome. This would be appealing, but is not essential for the concept of DNase II cleavage at a site of symmetry.

DNase II as a probe into conformational states of chromatin

In chromatin, the accessibility of the intranucleosomal cleavage site to DNase II correlates with a reversible transition from an extended to a condensed conformational state. In addition to the ionic environment, temperature has been found to affect the conformational state: splitting at the second site is virtually absent at 0 °C in nuclei but the accessibility increases with temperature until, at 37 °C, both sites are cut equally well⁷⁻⁸. In the presence of divalent cations, the temperature effects are not observed⁷. H1 is known to be involved in salt-induced aggregation of chromatin. H1-depleted calf thymus chromatin appears to have lost the intranucleosomal cleavage site, and this suggests that H1 is involved in the shift from the 200-nucleotide pair to the 100-nucleotide pair repeat pattern

(M. Steinmetz, R. E. Streeck and H.G.Z., manuscript in preparation).

Isolated oligomeric nucleosomes still have the ability to respond to ionic changes in DNase II digestion. They should therefore provide a system in which to study the parameters involved in this response. Detailed information on the effect of size and concentration of the isolated nucleosomes might tell to what extent aggregation or internal structural rearrangements of the nucleosome play a role. The results obtained with chromatin likewise do not enable us yet to decide if the opening and closing of intranucleosomal cleavage sites is due to conformational changes within the individual nucleosome or if it is the result of defined interactions between nucleosomal arrays, perhaps leading to a higher order packing of the chromatin fibril. It is clear, however, that nuclease digestion experiments can yield information not only on basic structural aspects but also on conformational states of nucleoproteins possibly related to higher structural orders.

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Thermal denaturation profiles and the structure of chromatin

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Analysis of the melting profile of DNA stripped of non-histone proteins and histone H1 allows definite structural conclusions to be drawn; conversely, it is possible to calculate what a particular chromatin model's melting curve will look like.

OPTICAL melting profiles of intact and partially stripped chromatin have been studied in many laboratories¹⁻⁵, and have been resolved into separate transitions, associated⁶ with free DNA and the DNA helices complexed with protein. It is

shown here that the shape of the melting profile can be calculated for a given model of the chromatin structure, and conversely that explicit structural conclusions can be drawn from the observed melting curves.

Theoretical considerations

The theoretical analysis makes use of the statistical mechanics of helix-coil transitions of two-stranded polynucleotides⁷. Neglecting the generally small effect of local heterogeneity of composition, one may divide the DNA helices into four classes, in respect of melting behaviour. These are illustrated in Fig. 1a and comprise (1) helix with free ends (*o,o*-helix), (2) helix joined at both ends to more stable helices (*h,h*-helix),

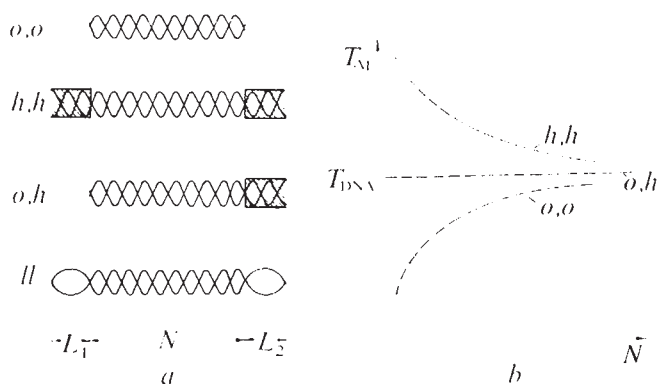


Fig. 1 *a*, Thermodynamically distinguished helix types: *o,o*, *h,h* and *l,l*-helices. *b*, Relationship between melting temperature T_m , and helix length N , for helices of *o,o*-, *h,h*- and *o,h*-type respectively.

(3) helix with one end free and the other joined to a more stable helix (*o,h*-helix), (4) helix between two loops, consisting of less stable, melted helices (*l,l*-helix). We now consider how the melting temperatures (T_m) of such helices depend on their lengths. Helices of the *o,o*-type have been fully treated^{8,9}: the T_m falls in a known manner with decreasing length. For an *h,h*-helix, on the other hand¹⁰, T_m rises with decrease in helix length. The other two types of helix have not previously been treated. The statistical mechanical analysis is given later.

The results are as follows: the melting of an *o,h*-helix is independent of its length; for an *l,l*-helix the situation is more complex; the relation between T_m and chain length depends on the size of the nucleotide loops at the ends. In the limit of zero loop length the configuration becomes equivalent to an *h,h*-helix, and as the loop grows, the T_m drops. It follows from the partition function (see statistics) that the T_m of an *l,l*-helix may be higher or lower than that of an infinite helix, but is always higher than that of an *o,o*-helix of the same length. The laws governing the melting of different types of helix are shown in Fig. 1*b*. A precise quantitative analysis is limited by lack of information about the magnitude of the helix initiation constant, σ , at low ionic strengths. At this stage therefore, only a semi-quantitative interpretation of the melting profiles can be attempted.

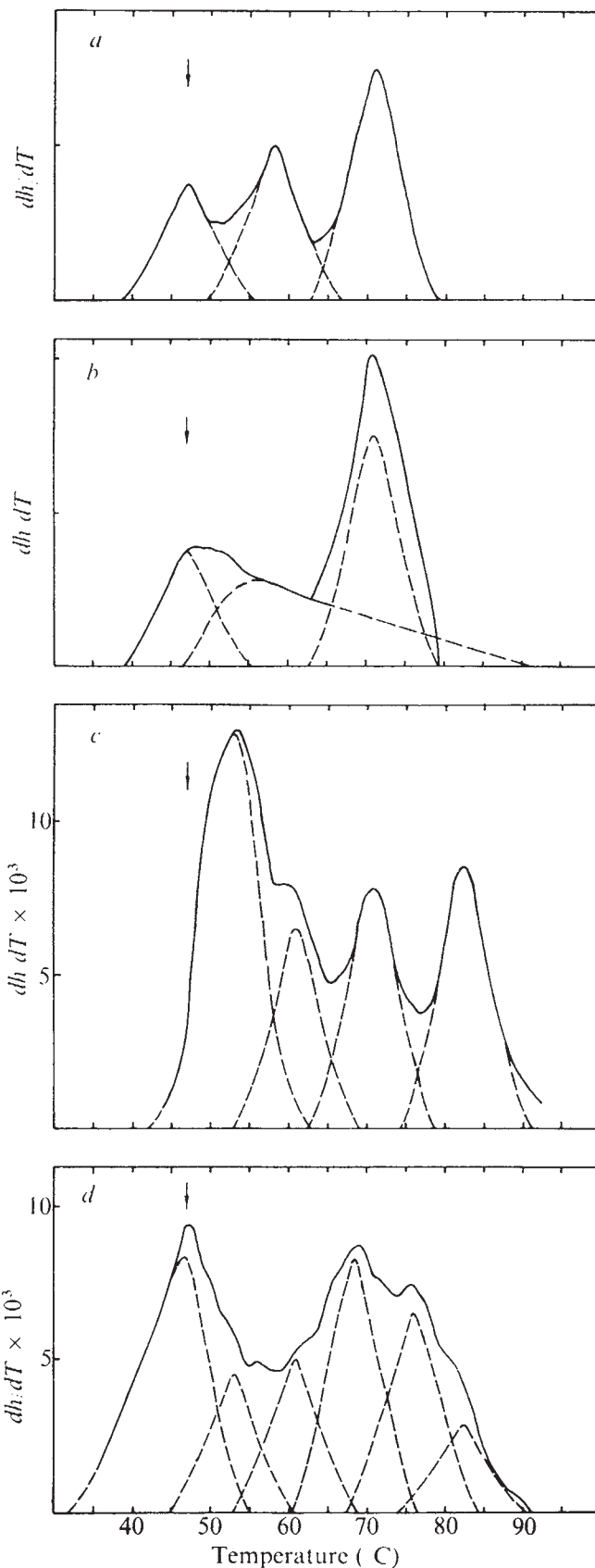
Chromatin models

The current view of chromatin structure¹¹⁻¹⁵ is based on a repeating unit of about 200 base pairs, 140 of these being in the form of a complex with protein (nucleosome), and the remaining 60 of a spacer of free DNA. On treatment with 0.6 M NaCl, the non-histone proteins and histone H1 are lost, but the periodic structure remains. In this material, called DNP 0.6, the nucleosomes contain only DNA and eight histone molecules, most probably two copies of each remaining species¹⁶⁻¹⁸.

We now consider the predicted melting behaviour of two possible models for DNP 0.6.

• **Equally Spaced Nucleosomes.** If the nucleosomes are all identical and spaced at equal intervals along the DNA, a melting profile encompassing three transitions is to be expected: that of naked DNA at the ends of the molecule (*o,h*-helices) with T_m as for free DNA (T_m^0); that of free DNA between nucleosomes (*h,h*-helices), with (as noted by Subirana⁹) $T_m > T_m^0$; and that of the nucleoprotein complex, which is associated with a higher numerical enthalpy of transition, both because of electrostatic stabilisation of the charged structure and the nature of the helix (*l,l*). The corresponding T_m will be higher than that of simple nucleosomes, in which the DNA has free ends. If the T_m of the second transition is sufficiently high, the second and third transitions may be brought into coincidence.

Fig. 2 Schematic derivative melting profile for a DNA duplex: *a*, with nucleosomes equally spaced along its length; and *b*, with randomly distributed nucleosomes; *c*, experimental profiles for DNP 0.6; and *d*, for the same material after sonication for 8 min at 1.2 A in an MSE sonicator. The DNP 0.6 was obtained from salt-extracted chromatin²⁰ after treatment with 0.6 M NaCl and chromatography on a Sepharose 4B column, 40×4 cm. Thermal denaturation profiles were measured in 0.25 mM Na EDTA, pH 8.0, with an instrument previously described^{21,22}. Differentiation was performed graphically, for intervals of 4 °C. The arrow corresponds to T_m for free DNA.



The breadth of the transitions will in the first place reflect the compositional heterogeneity of the duplex. The first and second transitions will in addition be broadened because of the finite lengths of the cooperative units, whereas the third phase (140 base-pair helix) should not be subject to broadening, and will resemble in shape the melting of free DNA. The areas under the peaks in the derivative melting curves will reflect the relative concentrations of the three types of structure. The predicted melting profile of this model for DNP 0.6 is shown in Fig. 2a.

• Randomly Spaced Nucleosomes. If alternatively the nucleosomes are randomly distributed along the DNA, the transition profile will be different (Fig. 2b). The second transition will be replaced by a continuous spread of melting of naked DNA. Segments short enough to have an intrinsic T_m greater than that of the nucleosomes will melt with the latter, since this implies a switch from h,h to l,l character, and the area under the corresponding peak in the derivative melting profile will be greater than would correspond to the DNA content of the nucleosomes. The third peak will be broadened by the distribution of loop lengths.

Analysis of melting profiles

The melting profile for DNP 0.6 is shown in Fig. 2c. There are four peaks, the three highest ($T_m = 61, 72$ and 82°C) corresponding to the most prominent components in whole chromatin, whereas the lowest ($T_m = 56^\circ\text{C}$) has no counterpart in chromatin. The areas under the peaks corresponding to the two highest temperature transitions correspond to the total protein content (see also ref. 3). Thus the first two transitions represent the melting of free DNA, and the other two that of complexed DNA. The first transition is broadened relative to that of free extracted DNA. The fourth is unchanged, and the second and third also do not seem to be significantly broader.

In analysing these curves we note first that there is no component of $T_m = T_m^0$, so that the amount of terminal naked

DNA is negligible. The presence of two peaks of melting of naked DNA with $T > T_m^0$ implies two populations of spacers (h,h -helices) between nucleosomes. The first and second transitions correspond to long and short segments of DNA helix respectively. The two transitions of protein-DNA complexes are sharp, and evidently represent standard, effectively homogeneous, populations containing relatively long segments of DNA with nucleotide distribution not significantly different from that of the whole DNA. Three possible reasons for the existence of these two latter (l,l -helix) transitions may be envisaged: (1) the presence of two regions differing in thermal stability within each nucleosome, (2) the existence of both long and short spacer regions between the nucleosomes; the latter would melt at temperatures determined by the length of the spacers which define the loop sizes, (3) the existence of two structurally distinct types of nucleosome.

We have found (to be published) that the relative areas under the two peaks in question depend strongly on the ionic strength, the sum of the two remaining constant.

This observation renders the first explanation improbable. It has been reported¹⁹ that mononucleosomes prepared by limited enzyme digestion melt in a single phase with a $T_m = 76^\circ\text{C}$; this is between the T_m values of the two l,l transitions. Since a l,l -helix necessarily melts at a higher temperature than the same helix in a o,o situation, the material melting in the 72°C peak cannot represent the same type of structure as that melting in the 82°C peak if both are normal nucleosomes. We therefore rule out the second explanation, to be left with the third.

The analytical procedures described above are strengthened by data obtained with ultrasonically fragmented material (Fig. 2d). The following features stand out:

• A new peak with $T_m = T_m^0$ (47°C) appears, which accounts for the melting of more than half the naked DNA. Thus the sonic fragments are short, consisting mainly of one to three nucleosomes.

Statistical mechanical analysis

The partition function Z for the ensemble of partially melted configurations of a double helix of N base pairs, in general depends on s the equilibrium constant for the addition of a base pair to the end of a helical region and σ the nucleation constant. In terms of the zipper model, whereby we ignore configurations with more than one helical region, we may write

$$Z(N) = \sum_{n=0}^N s^n w_n \quad (1)$$

where w_n is the statistical weight of configurations with n base pairs. The fraction of possible base pairs that are intact may then be written

$$f = \frac{1}{N} \frac{d \ln Z}{d \ln s} = \frac{\sum_{n=0}^N n s^n w_n}{\sum_{n=0}^N s^n w_n} \quad (2)$$

For helices in the o,h situation, w_n is unity for all N , since we assume that melting proceeds from one end of the helix only.

At the melting temperatures (T_m), $F_{o,h} = \frac{1}{2}$, and it may be shown that this occurs at $s = 1$; thus for o,h helices T_m is independent of N .

For helices in the l,l situation there are $N - n + 1$ ways in which the helix of n base pairs may be situated between the two loops. We assume that the two loops contain L_1 and L_2 nucleotides and write $L_1 = 2(N_1 - 1)$ and $L_2 = 2(N_2 + 1)$.

Each of the $N - n + 1$ configurations must be weighted according to the probability of ring closure. An approximation⁶ gives L^{-c} , where c is a constant, as the appropriate weight for a loop of L nucleotides. It then follows that

$$w_n = \sigma \sum_{k=1}^{N-n+1} \left[4(N_1 : N - n - 2 - K)(N_2 - K) \right]^c$$

for $1 \leq n \leq N - n + 1$

$$= 2^{-c}(N_1 - N_2 - N - 1)^{-c} \text{ for } n = 0$$

This expression may be substituted into equation (2) to yield $f_{l,l}$, which is clearly dependent on N , N_1 , N_2 , s and σ .

In the event that $L_1 = L_2 = 0$, however, the situation approximates to that of a h,h -helix (melting from both ends), and in the limit of very large L_1 and L_2 it approximates to the o,o situation. We therefore suppose that the plot of T_m versus N for a l,l -helix with finite loops would lie between the curves for h,h and o,o helices, at a position depending on the size of the loops. Consequently at constant N , T_m decreases with increasing size of the loops. It then follows that the T_m of finite helices in the l,l situation can be higher or lower than that of an infinite helix, but is always higher than the T_m of a helix of the same length but in the o,o situation.

• Concomitantly the other peaks associated with naked DNA decrease, showing that the intact DNP 0.6 contained no short segments of naked DNA, for otherwise sonication would have brought about an increase in the low-melting peaks.

• The peak of $T_m = 72^\circ\text{C}$ is shifted to lower temperatures by about 2.5°C , while the peak of $T_m = 82^\circ\text{C}$ is diminished by two-thirds in amplitude. At the same time a new peak appears at 76°C . The latter presumably corresponds to mononucleosomes²⁰. The transition at 82°C arises from the melting of nucleosomes in long fragments (*l,l*-helices), and that at 76°C from nucleosomes with free ends (*o,o*-helices). The peak at 72°C cannot be identified with a nucleosome in any state. It is present in DNP 0.6 but not in enzymically derived mononucleosomes. It may represent an altered or denatured nucleoprotein structure, which is not resistant to enzymic attack.

It is hoped to extend the quantitative scope of the analysis by determining the helix initiation constant, σ , at low ionic strength, which would allow a rather precise analysis of the lengths of the naked DNA segments in chromatin, and the evaluation of the thermodynamic characteristics of the nucleoprotein complexes.

The analyses of the melting profiles of DNP 0.6 allow the following conclusions to be drawn with considerable confidence: (1) DNP 0.6 consists of a long DNA molecule containing DNA-protein complexes separated by regions of naked DNA. (2) The melting temperatures of both naked DNA segments and DNA-protein complexes are not governed solely by their intrinsic thermodynamic characteristics (mean enthalpy and entropy), but depend strongly and in an interpretable manner on their lengths and positions in the structure. Earlier simplistic

interpretations of multi-phase transitions can no longer be sustained. (3) The melting transitions of naked DNA and of protein complexes (first two and second two peaks respectively) are completely separate. (4) The distribution of the nucleosomes along the DNA is independent of local nucleotide composition. (5) The material contains two different populations, each effectively homogeneous, of DNA-protein complexes, differing from each other in thermal stability and the mean lengths of the associated DNA.

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Electrified black holes

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Black holes with accretion disks are self-excited dynamos that generate large electric and magnetic fields. Along the spin axis they emit oppositely-directed narrow beams of high energy positrons and photons. The beams emanating from superholes are sufficiently powerful to explain extragalactic radio sources.

A BLACK hole has mass M , angular momentum J , and electrical charge Q , satisfying the relation

$$G^2 M^4 \geq J^2 c^2 - G M^2 Q^2 \quad (1)$$

where G is the gravitational constant and c is the speed of light. The equality sign denotes the maximum angular momentum and charge of the extreme Kerr-Newman metric. A spinning charged black hole has a magnetic dipole moment¹

$$\mathcal{M} = JQ/Mc \quad (2)$$

and at distances large compared with the gravitational length $R = GM/c^2$ the magnetic intensity obeys the familiar r^{-3} law. Normally, a large electric charge in a black hole would be rapidly neutralised by inflow of oppositely charged particles, and it has seemed reasonable therefore to suppose that charged black holes are not of great astrophysical importance. I shall attempt to show that a significant charge can in fact accumulate in black holes in quite general conditions, and that black-hole

electrodynamics may supply the long-sought-for explanation of the extended² and compact radio sources³.

Maximum electric charge

The maximum magnitudes of electromagnetic quantities, attainable in principle but not necessarily in practice, are denoted with a dagger. Thus a charge that contributes substantially to the mass of a black hole is approximately $Q_+ = G^{1/2} M$, and hence

$$Q_+/M \sim 10^{-18} e/m_p \quad (3)$$

where e/m_p is the charge-to-mass ratio of the proton. The corresponding electric potential is

$$\phi_+ \sim c^2/G^{1/2} \sim 10^{27} \text{ V} \quad (4)$$

and the electric field in the vicinity of a black hole is

$$E_+ \sim c^4/G^{3/2} \sim 10^{22} (M_\odot/M) \text{ V cm}^{-1} \quad (5)$$

(For $E_+ > 10^{16} \text{ V cm}^{-1}$, or $M < 10^6 M_\odot$, this field will break down spontaneously by the pair production of electrons.) The angular momentum that also contributes substantially to the mass is approximately $J_+ = GM^2/c$ and so the magnetic dipole moment is $G^{3/2} M^2/c$. In this case, the magnetic field intensity in the vicinity of the black hole is therefore

$$B_+ = E_+ \sim 10^{20} (M_\odot/M) \text{ G} \quad (6)$$

It is thus evident that black holes, in principle, can have the most intense macroscopic electromagnetic fields realisable in nature.

Gravity-induced electric charge

Eddington⁴ (see also refs 5 and 6) showed that stars are positively charged, and that electric fields play an essential role in their internal structure. Within a star—such as the Sun—proton and electron gases contribute equally to the pressure gradient, whereas gravity acts mainly on the relatively massive protons; an electric field therefore mediates between the proton and electron gases. In effect, electrons have velocities greatly exceeding the escape velocity, and some therefore escape leaving the star positively charged with an internal electric field that retains the remaining electrons.

Let ψ and ϕ be the gravitational and electric potentials. In a star like the Sun the forces acting on a proton and an electron are approximately equal

$$-m_p \nabla \psi - e \nabla \phi = -m \nabla \psi + e \nabla \phi$$

and hence, neglecting the electron mass, m , the electric field is

$$-\nabla \phi = \alpha(m_p/e) \nabla \psi \quad (7)$$

where $\alpha \approx 0.5$. In more massive stars α approaches unity owing to the pressure of radiation acting on the electrons. From the divergence of equation (7) it follows that the charge to mass ratio of a star is

$$Q_*/M = Gam_p/e \sim 10^{-36} e/m_p \quad (8)$$

where the gravity-induced positive charge Q_* is ~ 100 C/solar mass. The potential difference between the surface and centre of the Sun is $\sim 1,000$ V.

All self-gravitating systems containing free electrons have gravity induced positive charges of the order indicated by equation (8). The induced charge cannot be neutralised by the accretion of electrons nor can it be neutralised by surrounding a star with a negatively charged sheath. This is because the gravitational and electric fields exert everywhere a total force that acts equally on electrons and protons, and consequently an external ionised medium remains unpolarised. When Q is less (more) than Q_* the external medium is polarised and there is an outflow (inflow) of electrons until Q_* is restored and the external medium is depolarised.

Black holes that form by stellar collapse have initial charge to mass ratios of the order given by equation (8) and

$$Q_*/Q_{\dagger} \sim (Gm_p^2/e^2)^{1/2} \sim 10^{-18} \quad (9)$$

Their electric potential $\phi_* \sim Q_* c^2/GM$ is

$$\phi_* \sim m_p c^2/e \sim 10^9 \text{ V} \quad (10)$$

and is independent of mass. With angular momentum J_+ the magnetic field intensity is

$$B_* \sim 10^{-18} B_{\dagger} \quad (11)$$

or $\sim 10^3$ G in the vicinity of the black hole.

Superholes

Almost all matter in the Universe has too much angular momentum, either initially to form into black holes or subsequently to be accreted by black holes. Angular momentum must therefore be removed and, in the case of accretion, a viscous accretion disk develops in which gas spirals in toward the black hole and angular momentum is transported away⁷⁻¹⁰. A weakly charged black hole thus has angular momentum $J_{\dagger} \approx GM/c$ and approaches the extreme Kerr metric¹¹. An accretion disk generally lies in the rotational equatorial plane, and its orbital angular velocity has the same direction as the spin angular

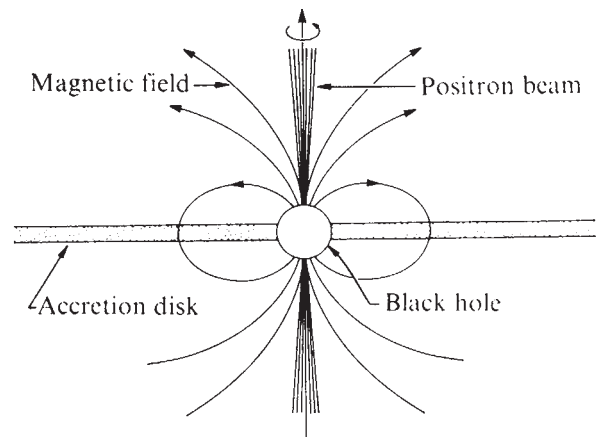


Fig. 1 A black hole with an accretion disk acts as a self-excited dynamo. The black hole rotates, it has an initial gravity-induced positive charge, and it generates a dipole magnetic field. The magnetic field induces a positive charge in the disk, and the charge accreted is therefore proportional to the charge already in the black hole. The charge in the black hole thus grows exponentially while its mass grows linearly with time. The production of high energy positron beams, that emanate from the polar regions, is discussed in the text.

velocity of the black hole. Black holes that form in matter-rich galactic nuclei accrete gas (also tidally disrupted stars) and become supermassive^{12,13}, attaining masses $\sim 10^8 M_{\odot}$ on a time scale $\sim 10^8$ yr. These supermassive objects are conceivably the primary sources of energy in quasars and in violent nuclear activity¹⁴.

A system consisting of a charged spinning black hole encircled by an electrically conducting accretion disk contains the basic elements of a self-excited homopolar generator. Such systems, if they actually exist, constitute the most powerful electrical machines in nature.

It is emphasised that throughout this treatment general relativistic subtleties are ignored, and all results are therefore only rude order-of-magnitude estimates.

Black-hole homopolar generator

A rotating accretion disk (see Fig. 1) of high electrical conductivity has an induced electric field

$$\mathbf{E} = -\mathbf{v} \times \mathbf{B}/c \quad (12)$$

where $\mathbf{v}$ is the orbital velocity and $\mathbf{B}$ is the axisymmetric magnetic field of the black hole.

The potential difference between the inner and outer regions of a disk in Keplerian differential rotation is

$$\phi \sim Qc^2/GM \quad (13)$$

and is of the same order as the Coulomb potential of the black hole. From the divergence of equation (12) the volume charge density in the disk is

$$\sigma = (\mathbf{v} \cdot \nabla \times \mathbf{B} - \mathbf{B} \cdot \nabla \times \mathbf{v})/4\pi c \quad (14)$$

Electromagnetic models in which trapped magnetic flux is accreted are considered elsewhere (R. V. E. Lovelace, unpublished; R. O. Blandford and R. L. Znajek, unpublished). The dragging inward of magnetic flux is ignored in this discussion and hence $\nabla \times \mathbf{B} \sim 0$. It follows, using cylindrical coordinates (r, ϕ, z) , that

$$\sigma = -(B_z/4\pi c) \partial(rv)/r \partial r \quad (15)$$

Since $v \propto r^{-1/2}$, and $\mathbf{B}$ in the disk is antiparallel to $\nabla \cdot \mathbf{v}$ for a positively charged black hole, the charge density is therefore also positive.

The basic mechanism of the proposed generator is that positively charged matter is accreted by a positively charged black hole. The induced charge σ in the disk is proportional to the charge Q in the black hole and therefore Q grows faster than the mass M . (The surface charge on the disk is determined by boundary conditions, and is not accreted.)

Let $\dot{M}$ be the rate that mass is accreted, and $\dot{Q}$ the rate that charge is accreted; further, let ρ_R and σ_R be the mass and charge densities of the innermost stable region of the disk close to the event horizon; then

$$\dot{Q}/\sigma_R \sim \dot{M}/\rho_R \quad (16)$$

where

$$\sigma_R \sim Q(GM)^{3/2}/8\pi c^3 R^{7/2} \sim Qc^6/8\pi G^3 M^3 \quad (17)$$

from equation (15). The mass density ρ_R is proportional to M/R^2c , and therefore

$$\dot{M}/\rho_R = G^2 M^2/\mu c^3 \quad (18)$$

where μ is plausibly ~ 10 . Equation (16), with equations (17) and (18), now becomes

$$\dot{Q} \sim Qc^3/8\pi GM\mu \quad (19)$$

and according to this simplified treatment the black hole charge grows exponentially on a time scale

$$T \sim 8\pi GM\mu/c^3 \sim 10^{-3}(M/M_\odot) \text{ s} \quad (20)$$

The initial 'seed' charge is the gravity induced value Q_* , and hence

$$Q \sim Q_* \exp(t/T)$$

Radiative discharge

In many respects, electrified black holes are different from pulsars. The spin and magnetic dipole axes of a black hole are coincident, whereas pulsars are oblique dipole rotators¹⁹. Pulsars have magnetospheres maintained by particle emission from the surface of a neutron star, and currents intersecting the surface enforce the magnetosphere into corotation¹⁶. The event horizon of a black hole cannot emit particles (quantum mechanical emission¹⁷ is negligibly small for the masses considered) and is not intersected by closed current loops. A black hole, therefore, is not embedded in a pulsar-like magnetosphere, and the huge potentials generated are therefore not easily short-circuited.

Electrons are attracted and accelerate along magnetic field lines that converge on the polar regions of the black hole. Let r_D be the outer radius of the disk; then the field lines not intersecting the disk converge on the polar regions at angles

$$\theta \lesssim (R/r_D)^2 \quad (21)$$

to the spin axis. The Larmor orbit radii of infalling electrons are small compared with R , and for R/r_D in the range 10^{-2} to 10^{-3} the electrons are confined to field lines of $\theta \leq 2^\circ$ – 6° . (The accreted electrons streaming down these narrow cones, in spite of space-charge limitation, may prevent the black hole from attaining large electrical potentials. Preliminary estimates indicate that this is unlikely.)

When the potential of the black hole is large, the infalling electrons emit curvature radiation that initiates a pair-production discharge^{18–20}. The created electrons and positrons of energy γmc^2 also emit curvature radiation consisting of photons of energy $\sim \gamma^3 \hbar c/R_c$, where R_c is the radius of curvature of the magnetic field lines. These photons cut across field lines and produce further electron pairs. All electrons plunge into the black hole and the positrons accelerate away in a narrow beam. If the photon mean free path, λ , for pair production is greater than a scale height $\sim R$ there is no significant discharge and the potential continues to rise. When $\lambda < R$, however,

pair-production avalanches until the black hole is discharged to the point where $\lambda \sim R$. With $\lambda \sim R \sim R_c$, the steady-state potential is found to be

$$\phi_s \sim 3 \times 10^{10}(mc^2/e)(M/M_\odot)^{1/2} \quad (22)$$

from formulae used in pulsar theory (refs 19, 20 and I. Lerche, unpublished). Thus a black hole of $10^8 M_\odot$ attains a potential 10^{20} V. The rate of converting gravitational energy into high energy particles is $\sim Q\dot{Q}c^2/GM$, or $\sim \phi_s^2 c/8\pi\mu$ from equation (19), and according to equation (22) the energy output in the beams is

$$L \sim 10^{37}(M/M_\odot) \text{ erg s}^{-1} \quad (23)$$

Accreting black holes emit low energy photons, and an alternative cascade mechanism is therefore possible (R. O. Blandford and M. J. Rees, unpublished). An infalling electron of energy γmc^2 inverse-Compton scatters a low energy photon to energy $\sim \gamma^2 kT$, where T is the temperature of ambient blackbody radiation²¹. The high energy photon now pair produces through photon-photon interactions, and the process avalanches if the photon mean free path, λ , is less than a characteristic distance $\sim R$. When $\gamma \lesssim mc^2/kT$, then $\lambda \sim (n\sigma_T)^{-1}$, where n is the blackbody photon density and σ_T is the Thomson cross section. In this case the vacuum breaks down at $\phi \sim 10^{12} T_4/T$ V when

$$M/M_\odot \lesssim 10^8 T/T_4 \quad (24)$$

and $T_4 \equiv 10^4$ K. Thus $10^8 M_\odot$ may be a limiting mass for the production of high energy beams. This type of breakdown, however, is not self-regulating; for if $\gamma > mc^2/kT$, then $\lambda \propto \gamma$, and the discharge quenches until the potential rises to the point where curvature radiation breakdown occurs in which $\lambda \propto \gamma^{-3}$.

Radio sources

Strong radio sources^{2,3} have energy outputs typically in the range 10^{42} – 10^{45} erg s⁻¹; a conspicuous characteristic feature is their double structure consisting of lobes of radio brightness extending in opposite directions from a quasar to an optically bright galactic nucleus; the extended double sources have estimated separations 10^5 – 10^7 light yr, whereas compact double sources have separations 10 – 10^2 light yr; also extended and compact sources often share the same optically bright central region, as in Cygnus A, and the radio brightness centres of extended and compact regions tend to be colinear. Various authors^{22–25} have advocated 'twin-beam' models for explaining double radio sources and shown that such models, in which oppositely directed beams of waves and relativistic particles created by quasars and galactic nuclei and lasting for 10^6 – 10^8 yr, have many attractive features.

The possibility that black holes have oppositely directed narrow beams of high energy particles is therefore of immediate interest in the study of radio sources. If it is granted that supermassive black holes with accretion disks exist, then we are provided with a natural mechanism that is capable of producing beams of sufficient power to excite radio sources. Positron-photon beams with entrained electrons will travel to great distances and energise the external magnetised medium. Thus, according to equations (22) and (23), superholes of mass $10^8 M_\odot$ emit high energy beams ($\gamma \sim 10^{14}$) with a power output of $\sim 10^{45}$ erg s⁻¹.

The specific properties of radio sources that can be explained by beamed emanations from electrified superholes are:

(1) Double structure: the two-component basic structure, often possessing remarkable symmetry, is the result of identical and oppositely directed narrow beams. Asymmetries and 'U' shapes (as in the 'head-tail' sources NGC 1265 and 3C 129 in rich clusters) are plausibly the result of density variations and relative motions of the intergalactic medium.

(2) Directional memory: Cygnus A and 3C 236 have compact double components aligned in the same direction as their extended double components, and the directional memory in these and other sources is provided by the spin axis of the

electrified superhole. Slow precession of the spin axis on a time scale of millions of years, owing to dynamic interaction with mass distributed in the nucleus, may explain the 'S'-shaped sources.

(3) Brightness distribution: the high energy beams progressively become more intense as the superhole grows in mass. So, in accord with observations²⁶, less luminous (therefore younger) sources have bright inner regions excited by greater beam intensity, whereas more luminous (older) sources have bright outer regions excited by beams that have attained great intensity over long periods of time.

Detailed investigations of electrified black holes will undoubtedly disclose more complex behaviours, such as, for example, non-quiescent discharges and beam pulsations on time scales $\sim GM/c^3$ of minutes to hours. These rapid fluctuations would be smoothed out in the extended radio brightness regions, but if 'core-halo' sources are two-component sources seen end on, the fluctuations might be detectable in the radio emission from the central milli-arcsecond structure of the core.

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letters to nature

Search for parity non-conserving optical rotation in atomic bismuth

THE Weinberg-Salam^{1,2} theory of the electromagnetic and weak interactions has been successful in predicting the neutral current interactions observed in neutrino experiments. An important further test for the theory is the experimental observation of predicted³ parity non-conservation in atoms. We are carrying out two related though distinct experiments to search for parity non-conserving optical rotation in atomic bismuth close to the allowed M_1 transitions from the ground state, $\lambda = 648$ nm (at Oxford) and $\lambda = 876$ nm (at Washington). Although our experiments are continuing we feel that there is sufficient interest to justify an interim report.

The predicted optical rotation angle close to the M_1 transition $i \rightarrow f$ is given by

$$\phi_p = \frac{-4\pi(n-1)LR}{\lambda}$$

where n is the refractive index and λ is the wavelength.

$$R = \frac{\text{Im}\langle f|E_1|i\rangle}{\langle f|M_1|i\rangle}$$

is the ratio of the parity non-conserving E_1 matrix element to the normal M_1 one. ϕ_p therefore has a dispersive dependence on frequency close to resonance. At resonance there is, of course, also absorption which limits the useful optical depth, $(n-1)L$, which can be used. For one absorption length at line centre the optical rotation at the dispersion peaks is $\pm R/2$.

A number of relativistic central field calculations of R have been made⁴⁻⁷ using both semi-empirical and Hartree-Fock methods. The ± 25 per cent agreement in the results is very satisfactory considering the differing starting points. We take

as average central field values $R_{876\text{ nm}} = -3 \times 10$, $R_{648\text{ nm}} = -4 \times 10^{-7}$, for a Weinberg angle $^{1,2} \sin^2 \theta = 0.35$.

The basic method used in the two experiments is the same: a brief description of the Washington experiment appeared recently⁸. In each experiment laser light at a frequency close to the M_1 transition is passed through two crossed polarisers between which is the bismuth oven ($T \simeq 1,500$ K). The transmitted light intensity has the form

$$I = A(\phi_p + \phi_M + \phi_R)^2 + B$$

where A and B are constants, ϕ_p is the optical rotation angle defined above, ϕ_M is an additional modulated angle produced by a Faraday cell and ϕ_R is any residual angle caused, for example, by misalignment of the polarisers. The basic signal, I_M , is that part of I which reverses with ϕ_M , normalised against changes in laser intensity and bismuth transmission. It is proportional to $\phi_M(\phi_p + \phi_R)$. The contributions from ϕ_p and ϕ_R are distinguished by the dispersive form of the rapid frequency variation of ϕ_p close to resonance.

There are a number of important differences between the experiments: (1) the pulsed parametric laser required at 876 nm has a linewidth too large to allow resolution of the individual hyperfine components which are separately observed in the 648-nm experiment. (2) The 648-nm line is overlaid by a molecular spectrum which limits the usable optical density to about 1 atomic absorption length. This difficulty is absent at 876 nm where an appreciably higher optical path length can be used. (3) Because the linewidth is much narrower in the Oxford experiment, the Faraday rotation in the bismuth caused by background magnetic fields is larger. To avoid the difficulty the oven is magnetically shielded and the experiment is designed to discriminate against bismuth Faraday rotations. (4) Largely because of the differences (1) to (3), different procedures have been adopted for picking out the sharp frequency dependence of the ϕ_p dispersion curve. In the Washington experiment this is achieved by repeatedly sweeping

the laser frequency through the resonance region while the signal I_M is accumulated in a multichannel analyser. The result can be fitted to the appropriate dispersion shape for several bismuth optical path lengths to subtract out the ϕ_R background. In the Oxford experiment the laser frequency is switched between two points of equal bismuth Faraday rotation, but opposite optical rotation. A small residual frequency dependent component of ϕ_R is subtracted out by repeating the experiment with a reduced bismuth optical path length.

Our results are as follows:

$$\text{Washington: } R_{876 \text{ nm}} = -8 \pm 3 \times 10^{-8}$$

$$\text{Oxford: } R_{648 \text{ nm}} = +10 \pm 8 \times 10^{-8}$$

where the quoted statistical error represents 2 s.d. There are, however, also systematic effects which we believe do not exceed $\pm 10 \times 10^{-8}$, but which are not yet fully understood. Within this systematic uncertainty the above results are each consistent with $R = 0$.

We conclude from the two experiments that the optical rotation in bismuth, if it exists, is smaller than the values -3×10^{-7} and -4×10^{-7} predicted by the Weinberg-Salam model plus the atomic central field approximation. Indeed Khriplovich⁷ has argued that the approximate theory used does overestimate R by a factor of ~ 1.5 . Lack of experience of this type of calculation means that more theoretical work is required before we can say whether or not the neglected many-body effects in the atomic calculation would make R consistent with the present experimental limits.

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General relativistic incompressibility

SIXTY years ago, Schwarzschild¹ developed what he, and others through the years, regarded as the general relativistic solution for a static, spherically symmetric, incompressible fluid sphere with equation of state $\rho = \rho_0 = \text{constant}$. Others have rejected the incompressibility interpretation of this solution including Eddington² who regarded $\rho - 3p = \text{constant}$ as the incompressible equation of state. We present here a new definition of incompressibility, consistent with basic principles of relativity. We then consider the natural generalisation of the Schwarzschild equation of state to general relativity, namely the constancy of proper density, and find that this approximates a truly incompressible fluid more closely than that presented by both Schwarzschild and Eddington. This could have interesting consequences in astrophysics since the maximum value of gravitational redshift, as well as the upper limit to the mass of a

neutron star, are larger for this model than for the Schwarzschild model, which is traditionally considered.

For the Schwarzschild solution, the upper limit for $2m/r_0$ is 0.889 because at this value the pressure becomes infinite at $r = 0$ (r is the Schwarzschild radial coordinate, m is the total energy of the body, $r = r_0$ at its surface and $G = c = 1$). Thus, for this case, the ultimate limit for static bodies, $2m/r_0 = 1$ cannot be approached. It has also been shown³ that under the seemingly natural restriction for normal bodies, $dp/dr \leq 0$, the maximum value for $2m/r_0$ which can be attained, irrespective of the equation of state, is 0.889, that is, in the Schwarzschild body. Moreover, this value corresponds to a maximum gravitational redshift of $z = 2$, and for some time, when the redshifts of quasi-stellar objects were piling up to $z = 1.95$, the situation was tantalising indeed. Since then, however, the z values of quasi-stellar objects have easily broken the $z = 2$ barrier. Also, ultradense matter raises the controversial question regarding the speed of sound exceeding the speed of light⁴⁻⁶.

In recent years, there has developed a growing realisation that $\rho = \text{constant}$ does not represent a constancy of physical energy density because of the role of gravitational binding energy⁷— ρ represents total energy density excluding gravitational energy.

True incompressibility, an idealisation in the realm of classical physics, is an even more remote concept in general relativity, because its realisation is already precluded in special relativity. Complete rigidity implies the instantaneous velocity of propagation of interactions. Instead, we propose the following definition of incompressibility.

A static, spherically symmetric body is incompressible if its equation of state is such that the pressure becomes infinite at $r = 0$ precisely when the limit $2m/r_0 = 1$ is reached.

Surely this represents the ultimate stiffness of matter which can be envisaged, and it can be envisaged only as a limit; it cannot be attained⁸, which is all to the good.

For physically reasonable equations of state, how close can matter come to being incompressible? We will consider this question in conjunction with our definition: how close can a static body come to having $2m/r_0 = 1$?

We first consider Eddington incompressibility. Letting $\rho - 3p \equiv B = \text{constant}$, we readily solve for one component of the metric

$$ds^2 = e^{\nu} dt^2 - e^{\lambda} dr^2 - r^2 (d\theta^2 + \sin^2\theta d\phi^2)$$

in terms of pressure. From the Einstein field equations

$$p' = -\frac{\nu'}{2}(B + 4p); \quad p = \frac{Ke^{-2\nu} + B}{4}$$

where the prime d/dr and K is the constant of integration.

Unfortunately, we then learn that the coupling of the equations for density and pressure which is demanded by the Eddington equation of state, leads to far more difficult differential equations for ν and λ themselves. The equation for ν is

$$CKe^{-2\nu} \left(\nu'' - (\nu')^2 - \frac{6\nu'}{r} - \frac{6}{r^2} \right) + CB \left(-\nu'' - (\nu')^2 - \frac{2}{r^2} \right) - \frac{4\nu'^2}{r^2} - \frac{4\nu''}{r^2} - \frac{8\nu'}{r^2} = 0; \quad C \equiv -8\pi G$$

This is to be compared to the Schwarzschild equation of state $p = \rho_0$ which yields

$$C\rho_0 r^2 = (e^{-\lambda} r)' - 1; \quad e^{-\lambda} = 1 + \frac{C\rho_0 r^2}{3}; \\ rDe^{-\nu/2} = e^{-\lambda} \nu' - (e^{-\lambda})'$$

With $e^{-\lambda}$ known, this easily leads to the solution for ν .

Nevertheless, we now have computers which were unavailable to Eddington. The numerical solutions have revealed that, at every stage, the Eddington equation of state corresponds to a body which is less stiff than the Schwarzschild body of equal mass and radius. This is consistent with Buchdahl's proof³ because

$$\frac{dp}{dr} = 3 \frac{dp}{dr} < 0$$

We feel that both the Schwarzschild and Eddington equations of state suffer from the deficiency of being too closely wedded to classical concepts. Relativistically, all energy, including gravitational energy, contributes to the total mass of a body, and in the extremely strong gravitational fields here considered, where bodies are close to their gravitational radii, gravitational energy is very significant indeed. In this respect, we now consider the natural general relativistic generalisation of the Schwarzschild equation of states.

The total relativistic mass of a body can be expressed as

$$M = \int_0^{r_0} \rho(r) 4\pi r^2 dr$$

This is found by matching to the exterior Schwarzschild metric. Since the integration is not over proper physical volume, clearly $\rho(r)$ does not represent proper energy density. If, however, we write

$$M = \int_0^{r_0} \frac{\rho(r)}{\sqrt{g_{rr}}} 4\pi r^2 \sqrt{g_{rr}} dr$$

we recognise $\rho_{\text{proper}} = \rho(r)/\sqrt{g_{rr}}$. In fact, in the limit of weak fields, it is readily shown that this modification accounts for gravitational binding energy⁷.

Thus, to generalise the Schwarzschild equation of state

$$\rho_{\text{proper}} = \frac{\rho(r)}{\sqrt{g_{rr}}} = \text{constant}$$

or

$$\rho(r) = Ae^{\lambda/2}; A = \text{constant}$$

In conjunction with the Einstein field equations, this yields

$$CAe^{\lambda/2} = e^{-\lambda} \left(\frac{1}{r^2} - \frac{\lambda'}{r} \right) - \frac{1}{r^2}$$

$$Cp = \frac{1}{r^2} - e^{-\lambda} \left(\frac{1}{r^2} - \frac{v'}{r} \right)$$

$$Cp = e^{-\lambda} \left[\frac{v'\lambda'}{4} - \frac{(v')^2}{4} - \left(\frac{v'}{2r} - \frac{\lambda'}{2} \right) - \frac{v''}{2} \right]$$

These equations were solved by numerical integration. The program was verified by solving the Schwarzschild equations, which led to results in agreement with the analytical solution to five significant figures. The results of the computer analysis of the equations are shown below. The equations were integrated outwards with infinite central pressure, and the radius of the body was determined by the condition that the pressure vanishes at the surface.

The graph compares pressure with e^λ rather than with radius because

$$(e^\lambda)_{\text{surface}} = \left(1 - \frac{2m}{r_0} \right)^{-1}$$

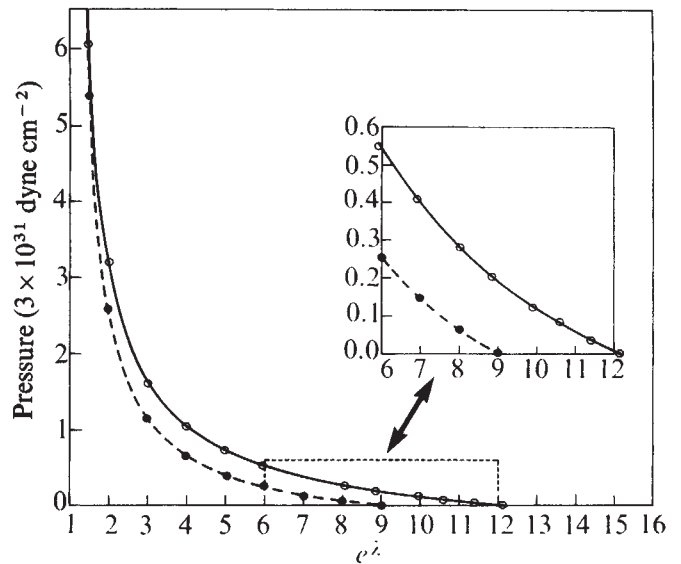


Fig. 1 Comparison of the behaviour of the constant $-\rho_{\text{proper}}$ solution (—) with that of the Schwarzschild solution (---).

yields $2m/r_0$ directly, obviating the necessity of integrating to find the total mass of the system. The Eddington solution has not been analysed for infinite central pressure. From our previous discussion, however, the Eddington equation of state corresponds to matter which is less stiff than that of Schwarzschild. From Fig. 1 $(e^\lambda)_{\text{surface}} = 12.1$ for ρ_{proper} constant, corresponding to the value $2m/r_0 = 0.917$. Thus, by our criterion, we have material which is stiffer than in Schwarzschild's and Eddington's work. Moreover, it implies a larger maximum gravitational redshift ($z = 2.48$) of spectral lines from the surface of a star. Although it represents an improvement over the Schwarzschild limit, redshifts have been observed to exceed this value⁸ and hence this alone would not suffice to provide an alternative hypothesis to the cosmological interpretation of QSOs.

Eddington's condition is equivalent to the classical concept of incompressibility because classically, for a homogeneous substance, mass and particle density are proportional. The Schwarzschild condition is a generalisation of this concept to special relativity since it takes into account changes in mass from motion, and the ρ_{proper} constant condition, incorporating gravitational energy as well, is a further generalisation of this same concept. It is not surprising, therefore, that among the three, the Schwarzschild fluid should be stiffer than that of Eddington, and the constant $-\rho_{\text{proper}}$ fluid stiffest of all.

As a final note, it is worth mentioning that Bondi⁹ went further still, achieving $2m/r_0 = 0.970$ under the assumptions $\rho > 0, p > 0$. His solution, corresponding to a "thin shell of matter containing a space stuffed full with density-free pressure", is, however, entirely unphysical.

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Is the velocity of P_n an indicator of Q_a ?

In a recent paper Booth *et al.*¹ showed that short period teleseismic P waves are recorded with relatively small amplitudes at long range seismic measurement (LRSM) stations in the western United States compared with those in the eastern half of the country. This is in agreement with the results of Jordan *et al.*² and several more recent studies. Booth concluded that this might be because the upper mantle anelastic quality factor Q_a is higher in the east, and consequently waves passing through it suffer less absorption.

The hypothesis has important consequences for the measurement of magnitude (m_b) and the proposal made by the United States and the Soviet Union to limit the size of their nuclear weapon tests stimulated further investigations of the idea. There is not an obvious way of doing so because opportunities to make reciprocal station-source experiments (firing at one station and recording at another, then firing at the second and recording at the first) are few. Would other geophysical quantities provide an indicator of Q_a in the upper mantle?

The velocity of the Moho refracted wave, P_n , is an obvious candidate; Herrin and Taggart³ have proposed that the variation in velocity of P_n might be due to fundamental differences between east and west in the upper mantle of North America. Evernden⁴ used P_n velocities to improve the estimates of magnitudes of near and near-regional earthquakes, and Alsop⁵ estimates Q_a in the uppermost mantle of the United States with the help of P_n waves.

The result of plotting Herrin and Taggart's observations³ of P_n velocity against the average short period P-wave amplitude residuals for stations overlying a particular P_n velocity is illustrated in Fig. 1. The two horizontal lines are simple averages. Cleary's⁶ data from the homogeneous (LRSM) network of stations are included with those of Booth *et al.* All five observations at 8.15 km s^{-1} are in Wisconsin and Minnesota where according to Herrin and Taggart, the P_n velocity is not well determined. (Note, however, that Smith *et al.*⁷ give a P_n velocity of 8.07 km s^{-1} for this area and that this eliminates the anomalous value.)

The correlation illustrated in Fig. 1 establishes an empirical relationship between the velocity of P_n and amplitude residuals

in North America. As it seems reasonable to suppose that the amplitude variations are caused by Q_a variation, we conclude that Q_a and the velocity of P_n are correlated. Archambeau *et al.*⁸ have already implied a relation between upper mantle P velocities and Q_a . The work which is developing from these key observations reported in this note indicates that such a relationship does exist, and that useful estimates of effective Q_a of the upper mantle and of amplitude (magnitude) residuals can be made wherever there are reliable measurements of P_n velocity. Since P_n velocities are available from most parts of the world it is possible to improve the accuracy of estimates of seismic yields of underground explosions. The more fundamental investigations into the physical significance of the observations are being pursued.

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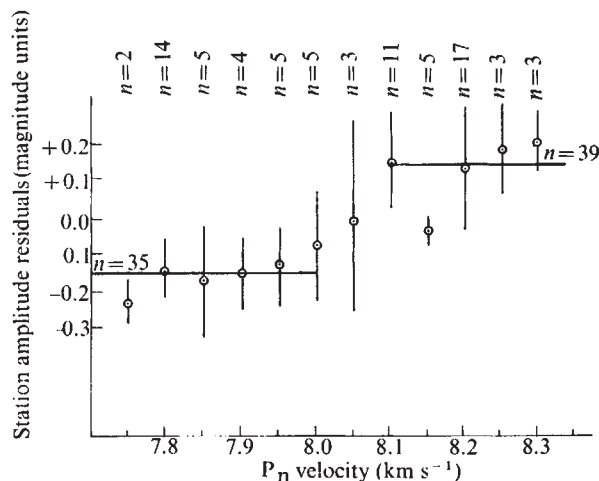
Use of phototransfer for the anomalous fading of thermoluminescence

THE occurrence of anomalous fading of thermoluminescence (TL) in minerals typical of the inclusions found in archaeological pottery has been reported¹. This effect leads to an erroneous evaluation of the archaeological age using conventional TL methods. Consequently it introduces serious problems in the dating of such materials. Here the phototransfer technique (re-excited glow)² was applied. The results indicate that in some materials anomalous fading may be circumvented, or at least substantially reduced. Of the materials of interest in TL dating that display anomalous fading (zircon, fluorapatite and plagioclase feldspars labradorite, andesine and bytownite), fluorapatite and zircon³ were found to be the most promising for the application of the phototransfer technique.

The samples tested (from the same specimen as used by ref. 1) were in the form of loose, crushed, grains and their TL was observed with apparatus that has been described by Aitken and Fleming⁴. Before the experiments these materials were annealed for 15 min at 700°C in an atmosphere of oxygen-free nitrogen. Ultraviolet illumination (usually 1-min exposure) of the samples was made at room temperature (RT) by means of a 300-W xenon lamp, the light from which was passed through a f/4.0 grating monochromator. A $^{90}\text{Sr}/^{90}\text{Y}$ β source was used for the irradiations.

The phototransferred TL (PTTL) was observed using the following procedure: first, irradiation at RT and subsequently erase TL to 500°C ; second, illumination with monochromatic light at RT and third the observation of re-excited TL by means of a glow curve of 500°C . The re-excited glow curves arising from phototransfer, obtained for fluorapatite and zircon are shown in Fig. 1. They are compared with the glow curves obtained after a short β

Fig. 1 Relationship between average station amplitude residuals and P_n velocity. Horizontal bars are obtained by summing all individual station amplitude residuals values $> 8.1 \text{ km s}^{-1}$ and all $< 8.0 \text{ km s}^{-1}$ and dividing by n the total number of observations in each velocity range.



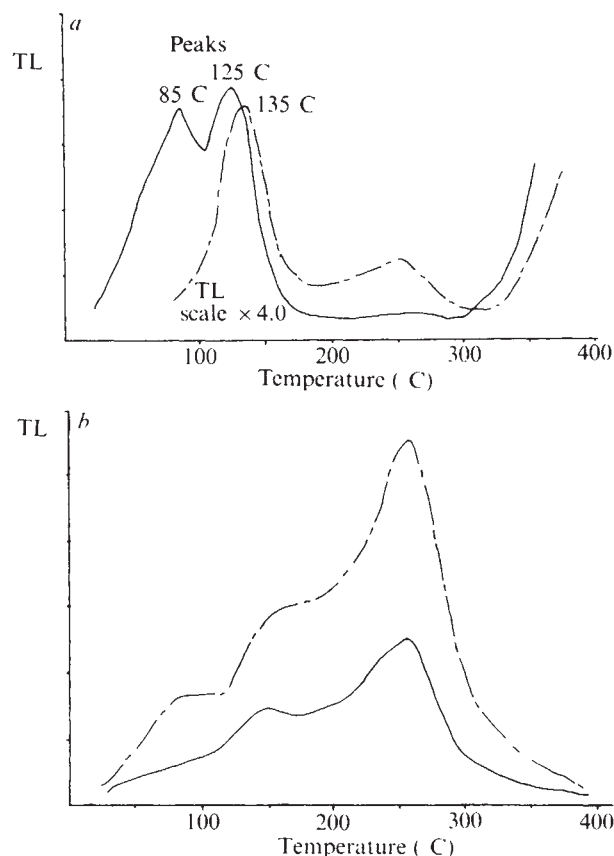


Fig. 1 TL glow curves: (a), zircon and (b) fluorapatite. In both *a* and *b*, — — — represents a typical PTTL glow curve, — — —, β -induced TL.

irradiation. In the case of zircon, the re-excited glow curve after ultraviolet illumination is different from that obtained after β irradiation; a peak is observed at 135 °C which is close to the dominant peak at 125 °C that is induced by β rays.

From the observations of PTTL following drainage to different maximum temperatures, donor levels (populated traps) have associated TL peaks in the region 500–700 °C in the case of fluorapatite and in the region 620–700 °C for zircon. We tested these donor levels for anomalous fading — samples of zircon and fluorapatite were given a dose of irradiation of ~25 krad and stored together with unirradiated samples for periods up to six months. The transferred TL from the irradiated samples was compared with the transferred TL obtained from the previously unirradiated samples that were β irradiated with the same dose just before measurement. Table 1 lists the results of the fading tests for the zircon and fluorapatite samples. In the case of zircon the donor levels associated with the 320-nm illumination were found not to fade and those levels associated with other wavelengths (280 and 240 nm) show some loss. Detailed investigation of the variation of transferred TL with wavelength of illumination⁷ indicates that there may be two types of centre involved in the transfer, and it appears that one of them does not fade.

Table 1 Long term fading test: % loss of PTTL (experimental error $\pm 3\%$)

Sample Illumination	Test period (months)	% loss transfer 320 nm	% loss transfer 280 nm	% loss transfer 240 nm
Zircon	6	0	15	20
Fluorapatite	3	3	No data	
	5	23		

The results for fluorapatite were less conclusive and the inconsistency (see Table 1) of 3% and 23% fading after 3 and 5 months storage respectively is to be investigated. As a comparison with conventional TL, tests for anomalous fading of the β induced glow curve (<500 °C) were carried out. For storage after irradiation (in the dark, at RT) for periods of 1 h (zircon) and 0.5 h (fluorapatite), the glow curves showed ~15% fading in the high temperature region (>150 °C) that is, where thermal untrapping had not occurred. Similar results were obtained with PTTL. The observations of the fading of the β -induced TL are in agreement with the findings of Wintle⁸, who has found that the anomalous fading of TL is not necessarily short lived; Fig. 2 shows results from her work for fluorapatite and zircon. These graphs indicate that, using conventional TL, extensive loss is to be expected for storage over a few months in the case of fluorapatite though less, but still serious, fading in the case of zircon.

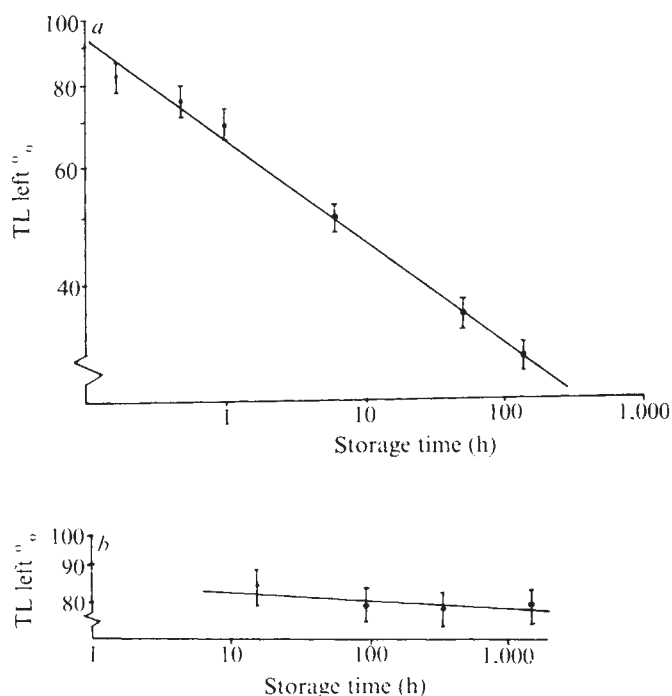


Fig. 2 Anomalous fading of TL. % TL left at 270 °C coordinate (fluorapatite (*a*)) and at 300 °C coordinate (zircon (*b*)) against storage time, at 10 °C (log/log plot).

For the purpose of dating, the growth in intensity of transferred TL with dose accrued by the sample (referred to as the 'growth characteristic') has also been investigated. Minerals such as zircon and apatites have high uranium and thorium content; typically 10^2 – 10^3 p.p.m. Consequently the effective internal radiation dose over archaeological time is⁷ in the region of tens of kilorads. For zircon and fluorapatite it has been found that the growth characteristic is linear up to 20 krad of accrued dose (see Fig. 3) for 320-nm illumination. Other wavelengths have also been investigated⁷. The non-zero intercept on the equivalent dose transferred axis results from a residual ultraviolet response, which in the case of zircon is significant. (The 'residual ultraviolet' excited TL refers to the glow curve obtained after ultraviolet illumination where the sample had not been irradiated since annealing to 700 °C.) Investigation of the wavelength dependence of PTTL and the residual ultraviolet excited TL indicates, however, that the situation should be improved for illumination with longer wavelengths. This and further work on the transfer properties of zircon and fluorapatite are to be reported elsewhere.

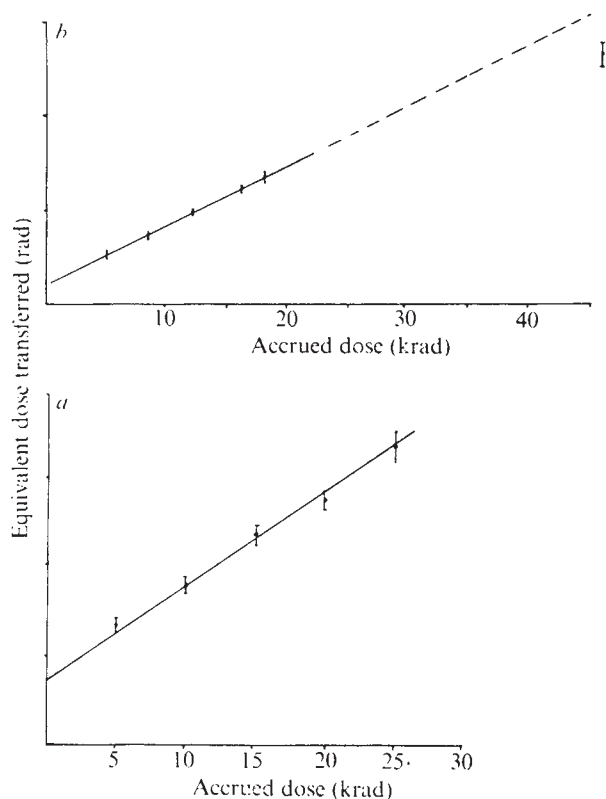


Fig. 3 Growth characteristics for (a) zircon and (b) fluorapatite under 320-nm (5 nm B-P) illumination. The 'equivalent dose transferred' (EDT) refers to the β dose required to be administered to the sample to produce a TL peak height that is equivalent to that of the PTTL peak. In the case of fluorapatite the 250 °C peak was used and for zircon the 125 °C β -induced peak was used to represent an EDT for the 135 °C ultraviolet-excited peak.

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Quantitative approach to molecular resolution electron microscopy

MANY attempts have been made to resolve individual atoms as ions, in molecules, and in crystals using conventional and scanning transmission electron microscopy^{1–5}. The results have been presented in the form of photographs for essentially qualitative assessment. In order to put the technique on a quantitative basis, we have analysed images of isolated molecules on thin supporting films using a digitising microdensitometer. We report here the results for a particular test molecule with a readily identified structure containing one heavy atom ($Z = 80$) for reference and two medium ones ($Z = 35$) for

determining the reliability threshold. As yet, images of lighter atoms ($Z = 16$) have been obtained only after extensive processing⁴, which impedes quantitative assessment.

We used a JEM 100 C microscope (JEOL Ltd, Japan) with voltage 120 kV, tilted beam dark field operation, objective aperture for spatial periods down to 0.24 nm, negative defocusing close to the Scherzer condition, and calibrated magnification 830 k. We chose Kodirex film (Kodak-Pathé, France) for the recording, as it has appropriate sensitivity, modulation transfer function (MTF), and detective quantum efficiency (DQE) characteristics. The specimen consisted of Merbromin ($C_{20}H_{10}Br_2HgO_6$) (K & K: ICN Life Science Group, USA) molecules deposited from solution in water on the support film. Figure 1 shows the probable structure determined by modelling: the separations of the mercury and bromine atoms are about 1.1, 1.0 and 0.6 nm. The support was a 400-mesh copper grid coated with Formvar, a holey carbon film, and an ultrathin (≤ 1.5 nm) amorphous boron layer. We found that the boron layer was stronger and more uniform over the holes than carbon ones, and gave less background noise. Bromine atoms are usually sufficiently well resolved against uniform regions of the support film to present few problems. We deliberately chose a molecule close to a non-uniform region in order to demonstrate the danger of interpreting from 'improved' photographs, and to demonstrate that the quantitative analysis of the original recording yields a measure of its reliability. Figure 2 shows two enlargements made from the same part of the original. Fig. 2a was printed conventionally. (The process could not resolve the low contrast details visible in the negative.) Fig. 2b was obtained by copying with four times magnification on high gamma duplicating film and then printing on hard paper. It reveals the bromine atoms;

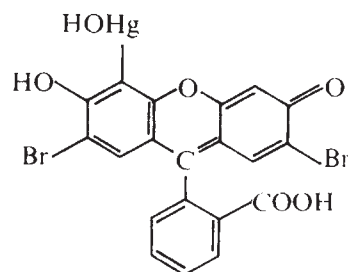
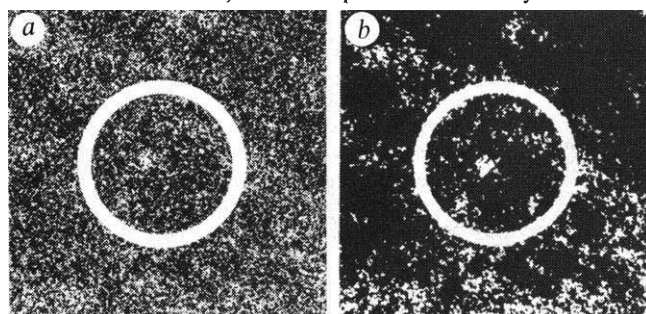


Fig. 1

but the granular structure and the lack of intermediate tones makes it difficult to distinguish the molecule from the noise. There are other processing methods, which yield cleaner pictures⁴; but the probability of misinformation is greater.

In our quantitative approach, we first examined the original recording using a low magnification optical microscope with numerical aperture sufficiently large for the range of spatial periods. We then evaluated likely regions using a microdensitometer. This was a flat-bed model which

Fig. 2 Triad of mercury and bromine atoms in merbromin molecule; b has been processed for clarity.



1 nm

$M = 10 \times 10^6$

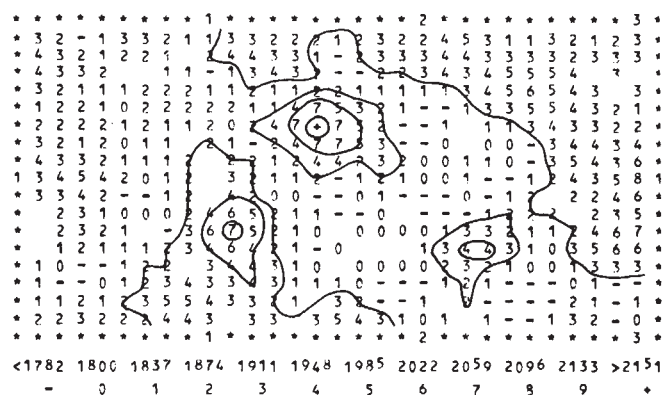


Fig. 3

had been modified in the laboratory for scanning and digitisation. A $(25\text{-}\mu\text{m})^2$ sampling aperture yielded reasonably constant MTF for spatial periods down to $\sim 0.15\text{ mm}$, and that we found adequate for Kodirex. We chose the sampling step somewhat smaller than half the minimum spatial period for the microscope, $80\text{ }\mu\text{m}$ corresponding to 0.096 nm . The data were tabulated by computer. A crude contour map is shown in Fig. 3: each number indicates the highest of ten contour levels which passes within half a spacing of a grid point. (We found it necessary to use a second evaluation for the map with a larger sampling aperture so that the background fluctuations were reduced by MTF roll-off.) The data from single line scans through the atoms are shown in Fig. 4. We considered it inappropriate to fit curves through the points, as that was not particularly informative in view of the sampling process. We also found that the measured optical densities, D , were not very helpful, and we decided to refer the values to the mean $\bar{D}$ (horizontal line) and the root mean square fluctuation $\bar{D}$ for background scans on either side of the molecule. The resulting local signal-to-noise ratios $(D - \bar{D})/\bar{D}$ were 3.8 and 2.0, 1.8 for the mercury and bromine atoms. The mean Hg/Br signal ratio for several evaluations with different specimens was 1.9, suggesting reasonable prospects for heavy/light atom discrimination. The variance within the (admittedly) small sample was disconcerting, implying poorer reliability than we had supposed from the visual assessment.

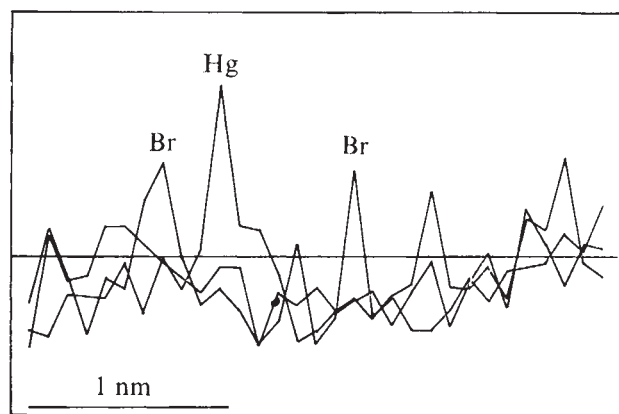


Fig. 4

The scan profiles can be misleading: for example, the peaks to the right of the molecule may be interpreted as other atoms. Careful analysis of the patterns in two directions normally separates the useful information from the "noise"; but that clearly relies on previous knowledge of the structures. The widths of the profiles are no guide for the resolution attainable. We believe that the minimum workable separation is significantly smaller than twice the limiting spatial period; but, unfortunately, it is certainly larger than the atomic bond distances. (We are now trying molecules with smaller spacings.)

We are sufficiently encouraged by the results for uranium, mercury, barium, iodine, strontium⁵ and bromine atoms to continue the search downwards. In view of the background fluctuations, we are doubtful of the prospects for light atoms, but we remain hopeful for chlorine ($Z = 17$). In another direction, we are collaborating in an application to tag subunits of large biomolecules. We are also developing more practical techniques of image analysis using a minicomputer with a graphics display terminal.

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Low temperature specific heat of glasses and amorphous solids

IN a previous paper¹ we attributed the anomalous low temperature thermal properties of amorphous materials to the inherent nature of the amorphous state, conceived of as the crystalline state saturated with the most favoured dislocations². Using Granato's vibrating string model³ of a dislocation, an estimate of the excess specific heat of $\text{Pd}_{0.775}\text{Cu}_{0.06}\text{Si}_{0.165}$ was provided. The purpose of the present note is to clarify several points in our earlier paper.

The experimental value^{4,5} of the specific heat of glassy $\text{Pd}_{0.775}\text{Cu}_{0.06}\text{Si}_{0.165}$ previously quoted is actually the electronic specific heat of the crystalline solid and not the linear contribution associated with the amorphous state. For the anomalous linear region of amorphous $\text{Pd}_{0.775}\text{Cu}_{0.06}\text{Si}_{0.165}$ the data of Golding, Bagley and Hsu⁴ indicate an upper limit of $C_v/T \approx 1.1 \times 10^4 \text{ erg mol}^{-1} \text{ K}^{-2}$.

As the previous calculation for the linear specific heat of amorphous $\text{Pd}_{0.775}\text{Cu}_{0.06}\text{Si}_{0.165}$ used weighted averages for the parameters involved, it is felt that a revised calculation using experimentally determined values is in order. The pertinent values determined by Golding, Bagley and Hsu⁴ are $\rho = 10.52 \text{ g cm}^{-3}$, $G = 3.48 \times 10^{11} \text{ dyne cm}^{-2}$, and $\theta = 252 \text{ K}$. Noting that the saturation condition corresponds to a dislocation density $\Lambda = (2r_c^2\sqrt{3})^{-1}$ where r_c is the core radius, then saturation occurs for $\Lambda = 7.9 \times 10^{13} \text{ cm}^{-2}$ (a weighted average of $4.1 \times 10^{-8} \text{ cm}$ has been assumed for the lattice parameter). Substituting the above values into the expression for the dislocation contribution to the specific heat.

$$C_v = \frac{\rho \pi^2 \Lambda a^2 N k}{3} \frac{T}{\theta} \quad (1)$$

now yields $C_v/T = 8.6 \times 10^4 \text{ erg mol}^{-1} \text{ K}^{-2}$, as compared with the experimental value of $1.1 \times 10^4 \text{ erg mol}^{-1} \text{ K}^{-2}$. It must be noted that this is an absolute upper limit on the calculated dislocation specific heat contribution.

Lu and Nelkin⁶ have also examined the low temperature thermal properties of glasses in terms of dislocations, indicating that a dislocation density of 10^{10} – 10^{11} cm^{-2} is required to

explain the linear specific heat (this calculation employed the Lifshitz-Kosevich⁷ model of an edge dislocation) and further speculating that the tunnelling of dislocation cores between equilibrium configurations may be responsible for the phonon scattering (a similar idea has been advanced by Anderson⁸ for a sessile dislocation in a complex lattice). Tunnelling of the cores would be consistent with the two-level systems postulated in the tunnelling states model^{9,10}.

There are difficulties associated with the concept of dislocations in glasses, the validity of the string model at these high densities, and the behaviour of the strain fields at this density. These are all questions which need to be addressed. But Ashby and Logan¹¹ have pointed out that within the framework of the random-network model "dislocation-like" features are possible in glasses and that they may have a very high density. In earlier work Scott and Giles¹² have succeeded in explaining the thermal conductivity of Teflon from 0.17 to 4.0 K on the basis of dislocation scattering of phonons. Further, small-angle X-ray scattering experiments are not inconsistent with the presence of dislocation strain fields in glasses⁶. Caution should also be exercised in strict application of the string model to explain both the specific heat and thermal conductivity of glasses. But in view of the rather interesting, although speculative, implications of these ideas, we feel that the concept of dislocations in amorphous solids is worthy of further examination, especially its compatibility with the tunnelling states model.

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Fig. 1 View of venting St Augustine volcano as the aircraft lines up for a plume penetration.

1976), taken as the National Center for Atmospheric Research (NCAR) Electra aircraft (instrument boom visible to left) prepared to enter the plume on a sampling run, is shown in Fig. 1. During this and other sampling runs ice nuclei in the air were captured on Millipore membrane filters using the NCAR SAMOVAR Air Filtration System⁶.

To reduce the analytical uncertainty that seems to be associated with all methods of membrane ice nucleus analyses, rather than filtering aerosol through single 3.7- or 4.7-cm diameter filters as is commonly done, an array of filters was produced by first exposing a single 28.7-cm square, 0.45 μm pore diameter, Millipore filter supported by a fibre glass backing. This large filter was then sectioned with a circular stainless steel die to produce 25 separate 4.7-cm diameter filter disks all exposed in the same volume of air. Each of the segments is considered to be equivalent to each of the others. Between 0.5 and 5.0 m^3 of air flowed through each filter disk at a rate of 0.15 $\text{m}^3 \text{min}^{-1}$.

One part of the experiment consisted of exposing a large Millipore filter 800 km upwind of the volcano over the Gulf of Alaska in marine air far removed from volcanic and human influence. This air was considered to contain a representative background ice nucleus population for the air subsequently moving on to the coast of Alaska. The first exposure was followed by exposure of a new filter totally within the plume of the volcano on the following day. Other measurements included exposure of a filter in background air and re-exposure of the same filter within the plume. This sequence was followed to observe whether plume effluents deactivated natural ice nuclei. Yet another filter was exposed solely in plume air 16 d after the second major eruption.

The Millipore filter sections were processed in a thermal diffusion chamber identical to the one described by Langer and Rodgers⁷, except for the water vapour source for these studies which consisted of three thermally controlled ice-covered plates instead of ice cubes. From each large filter array, five filter disks were processed at -10 , -15 , and -20 $^{\circ}\text{C}$. To calibrate the system to operate at water saturation, or possibly slight supersaturation, the filter substrate and ice temperatures were adjusted so that a fog, and eventually droplets, appeared on aluminium foil placed in the processing chamber. Further evidence of proper vapour control was seen when water-depleted 'halos' formed around growing ice crystals on the filters being processed; thus it may be suggested that condensation-followed-by-freezing may have been occurring in addition to nucleation by deposition. Typical ice crystal counts per filter were 1–20 at -10 $^{\circ}\text{C}$, 9–90 at -15 $^{\circ}\text{C}$, and 200–800 at -20 $^{\circ}\text{C}$ before

Airborne ice nuclei near an active volcano

ACTIVE volcanoes have been suggested as being sources of atmospheric ice nuclei^{1,2}, the rare yet crucial particles that initiate much of the Earth's precipitation. Other studies indicate that some volcanoes are not adding ice nuclei to the atmosphere or may be deactivating natural ice nuclei³. We report here measurements of atmospheric ice nucleus concentrations upwind and within the effluent plume of St Augustine, an actively venting island volcano situated at the base of the Aleutians in the mouth of Cook Inlet, Alaska ($59^{\circ}20' \text{N}$, $153^{\circ}30' \text{W}$). St Augustine erupted violently on January 23, 1976 and then again on February 4, 1976. We find no evidence for ice nuclei production by this volcano. Between and after the major eruptions the volcano actively vented giving off gaseous and particulate matter. Sulphur dioxide concentrations in the plume were >5 p.p.m. (J. Moyers, unpublished) and the total particulate loading was $\sim 100 \mu\text{g m}^{-3}$ (W. Zoller, unpublished) during the venting stages. A photograph of the venting volcano (February 1,

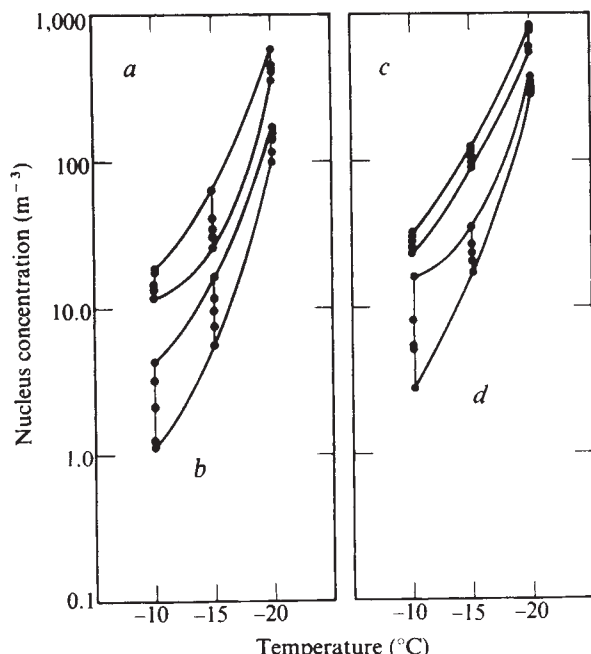


Fig. 2 Atmospheric ice-nucleus concentrations measured upwind and within the plume of erupting St Augustine volcano, Alaska. The solid dots represent the ice nucleus concentrations observed; the trend lines serve merely to bracket the separate ice nucleus concentration sets. *a*, Gulf of Alaska, January 31, 1976; *b*, plume, February 1, 1976; *c*, background and plume, February 1, 1976; *d*, plume, February 20, 1976.

subtraction of background counts (observed on unexposed filters) of 9, 6, and 45 crystals, respectively. The resultant crystal counts were corrected for volume effect⁸ and then normalised to a unit volume of air.

Results from analyses of four filter arrays are shown in Fig. 2 where it can be seen that the air over the Gulf of Alaska sampled on January 31, 1976 (*a*) contained more ice nuclei than did the volcanic plume sampled on the following day, February 1 (*b*). Comparisons between the ice nuclei in the plume air on February 1 (*b*) and the ice nuclei observed on filters exposed to background air followed by re-exposure to plume air (*c*) indicate that slightly higher ice nucleus concentrations were observed in the latter samples. The plume air of February 20 (*d*) contained ice nuclei in concentrations slightly in excess of the background air measured over the Gulf of Alaska (*a*).

All of the ice nucleus measurements shown in Fig. 2 fall within the range of 'typical' ice nucleus spectra observed in 'background' air at other locations around the globe⁹. The concentrations of ice nuclei active at -20°C in the plume on February 20 (*d*) (highest concentrations observed) fall between 560 and 920 nuclei m^{-3} . These values bracket the measurement of 0.7 nuclei l^{-1} active at -20°C measured in the plume of venting Mt Baker (Washington) during March and June 1975 as reported by Radke *et al.*¹⁰.

We suggest, on the basis of our measurements, that the effluent plume of St Augustine was not contributing significantly to background atmospheric ice nucleus concentrations on the days studied. There is some suggestion that the effluent gases may be deactivating natural ice nuclei in some cases as evidenced by relatively low ice nucleus concentrations measured on February 7. If this is so, this deactivation is relatively small compared with the inherent variability of natural atmospheric ice nucleus concentrations generally observed in the atmosphere¹¹. This variability could also explain these lower measurements. Since the particulate output of all the Earth's volcanoes (actively erupting and venting) is estimated to be ~ 0.005 of the total Earth-derived aerosol load¹¹, it is further suggested that

volcanoes venting similarly to St Augustine are probably exerting little influence on local atmospheric ice nucleus concentrations and negligible effects on global atmospheric ice nucleus concentrations. We anticipate measuring ice nucleus concentrations within the plume of an erupting volcano in the coming years.

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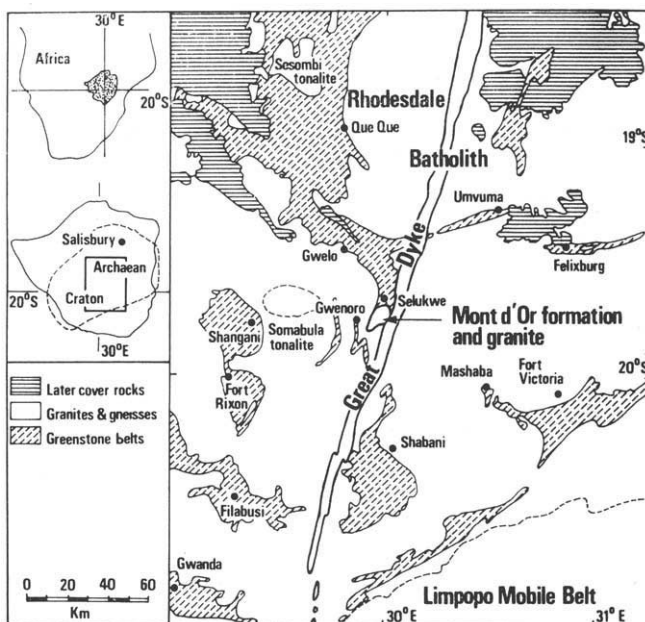
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Early Archaean age for the Sebakwian group at Selukwe, Rhodesia

WE present here the results of a Rb-Sr age and isotope study of granite (about 70% SiO_2) from the north-eastern sector of the Mont d'Or formation in the central part of the Rhodesian Archaean craton (Fig. 1). A nine-point Rb-Sr whole rock isochron (Fig. 2, Table 1) yields an age of $3,420 \pm 60$ Myr, with an initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.711 ± 0.001 (both errors at 1σ). We regard this as the age of intrusion.

The current nomenclature for the subdivisions of Rhodesian greenstone belts invokes three informal lithostratigraphic 'groups', the Sebakwian, Bulawayan and Shamvaian.

Fig. 1 Geological sketch map of part of the Rhodesian Archaean craton, showing locality of Mont d'Or granite.



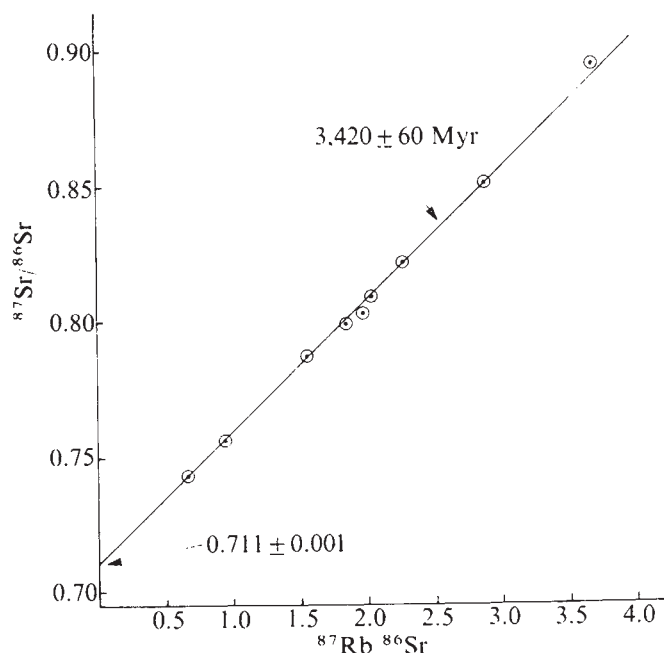


Fig. 2 Rb-Sr whole rock isochron plot for Mont d'Or granite samples. Errors are quoted at the 1 σ level. Error factor required to achieve perfect least squares fit is 2.5. Decay constant for ^{87}Rb is $1.39 \times 10^{-11} \text{ yr}^{-1}$.

The Sebakwian is the oldest, with the younger Bulawayan and Shamvaian groups together constituting the main greenstone belts¹. Age measurements on volcanic rocks from the Bulawayan group indicate an age of eruption of about 2,700 Myr (ref. 2). The Selukwe greenstone belt is assigned to the Sebakwian group on the latest (1971) edition of the geological map of Rhodesia issued by the Rhodesia Geological Survey. The Selukwe area is unique in that the succession is inverted and forms part of the lower limb of a large recumbent fold³⁻⁷. This structure is called the Selukwe nappe.

Table 1 Rb-Sr analytical data for Mont d'Or granite

Sample no.*	$^{87}\text{Rb}/^{86}\text{Sr}^\dagger$	$^{87}\text{Sr}/^{86}\text{Sr}$	Rb(p.p.m.) ‡	Sr(p.p.m.) ‡
1,167	2.04	0.8083 $\pm$ 1	71	101
1,204	1.97	0.8010 $\pm$ 2	66	97
1,223	1.55	0.7873 $\pm$ 1	51	95
1,249	2.88	0.8514 $\pm$ 1	100	102
1,263	1.85	0.7982 $\pm$ 1	96	151
2,123	2.27	0.8218 $\pm$ 1	110	142
2,974	3.68	0.8949 $\pm$ 1	65	52
3,692	0.659	0.7431 $\pm$ 1	42	185
3,724	0.951	0.7561 $\pm$ 1	53	162

*From borehole S636, depths in feet.

† Determined by X-ray fluorescence¹⁰; average precision $\pm$ 1%.

‡ Mass absorption coefficients estimated from background; concentration data $\pm$ 5%.

The Mont d'Or formation consists largely of quartzofeldspathic rocks containing chlorite and sericite. The sericitised nature of many of the rocks has led to dispute concerning the original nature of the formation. Stowe³⁻⁵ favours a largely sedimentary origin and regards the Mont d'Or formation as the lowest (oldest) stratigraphic unit in the nappe structure, but describes the presence of later, small, irregular intrusive bodies of granite. Cotterill⁷, on the other hand, considers that the greater part of the formation consists of a granite which is intrusive into the nappe structure. He recognises meta-sedimentary enclaves within the granite, but regards them as lateral equivalents of the Wanderer formation, the major sedimentary unit within the nappe succession (Table 2).

The difference in interpretation is, in effect, largely one of amount of later granite present. Our samples come from a deep borehole (S636) from the northern part of the area underlain by Mont d'Or formation where both authors agree to the presence of later granite. Thin sections of our samples show quartz, potash feldspar, sodic plagioclase, a small amount of chloritised biotite, and variable sericitisation of the feldspars.

Our results demonstrate a pre-3,400 Myr age not only for the Selukwe greenstone belt (Sebakwian group), but also for the nappe structure itself.

Table 2 Main stratigraphic units of the Selukwe greenstone belt, youngest formation at the top

After Stowe ⁵	After Cotterill ⁷
Small bodies of late granite	Mont d'Or formation (dominantly granite) with intrusive contact
Nappe tectonics	Nappe tectonics
Upper Greenstone formation	Tibilikwe formation (metabasalt with minor banded iron formation)
Wanderer formation	Wanderer formation (conglomerate and grit, overlain by banded iron formation)
Unconformity	Unconformity
Lower Greenstone formation (with serpentinites)	Selukwe Greenstone formation intruded by Selukwe ultramafic formation
Mont d'Or formation (dominantly meta-arenites)	

The eastern side of the Selukwe greenstone belt is cut by the Great Dyke (~2,500 Myr). East of the Great Dyke, the metasediments and chromite-bearing ultramafic rocks of the extension of the Selukwe greenstone belt occur tightly folded with northerly trending banded gneisses and migmatites. Very similar rocks, again assigned to the Sebakwian group, and also tightly folded with gneisses on a distinct northerly trend, occur farther east in the Mashaba area⁸. Gneisses from the Tokwe and Shashe rivers near Mashaba have yielded a rather poorly defined age of $3,580 \pm 200$ Myr (ref. 2). East of Mashaba, the Mushandike granite, which intrudes similar gneisses, gave an age of $3,520 \pm 130$ Myr (ref. 9).

Thus our results from the Mont d'Or formation granite are in keeping with the regional picture of a ~3,500–3,600 Myr granite-gneiss terrain between Selukwe and Mashaba. In addition, they provide confirmation of the inference by Hawkesworth *et al.*² that the greenstone belt remnants within this terrain are also ~3,500–3,600 Myr old. The Selukwe greenstone belt is thus the largest preserved belt of this age known in Rhodesia. The possibility also arises that the Selukwe nappe structure is related to the tectonism which produced the prominent northerly trends in the gneisses between the Great Dyke and Mashaba.

The initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.711 ± 0.001 of the Mont d'Or granite is, as far as we know, the highest value yet recorded for such an ancient granite. The initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios for the ~3,500–3,600 Myr gneisses mentioned above are within the range 0.701–0.702. We provisionally interpret the high initial ratio of the Mont d'Or granite as indicating partial melting of earlier sialic crust, which need not necessarily be very much older. Thus, 3,600-Myr-old gneisses from the Shabani area, some 70 km S of Selukwe, have sufficiently high Rb/Sr ratios for their average $^{87}\text{Sr}/^{86}\text{Sr}$ ratio to increase by about 0.005 to 0.010 in as little as 200–300 Myr (S.M. and J.F.W., unpublished). It is possible that the Selukwe area is also underlain by ancient, high Rb/Sr crust of this type.

There is clear evidence for earlier granitic rocks in the pre-Mont d'Or Wanderer formation. Stowe³⁻⁵ and Cotterill^{6,7} record boulders and pebbles of various types of granite and gneiss in the conglomerate of this formation.

These could have been derived by erosion of 3,500–3,600-Myr-old basement similar to that exposed further to the south and east.

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Monitoring the marine environment for mutagens

VARIOUS chemicals found in the environment are mutagenic when tested in an appropriate screening system¹, and some of them are deposited as waste in the seas and oceans, where they may or may not be deactivated. A means of monitoring the marine environment for the presence or absence of mutagens would give some indication of the efficiency of disposal of industrial waste products. In an attempt to minimise the technical problems, we have developed a biological assay for the detection of such pollutants.

Our assay detects mutagens in the tissue of the mussel *Mytilus edulis*, which is widely distributed in the tidal zone

and shallow coastal waters of the northern and southern hemisphere, and has been used in several studies of water pollution and interspecific relationships^{2–5}. Because of its mode of filter feeding and intracellular (phagocytic) digestion, this organism may concentrate marine pollutants within its body tissues and these provide a source of testable material.

Test samples were screened using auxotrophic yeast cultures to detect the induction of prototrophic colonies produced by mitotic gene conversion, a process of genetic change that responds to treatment by mutagens and carcinogens in a nonspecific manner⁶. Extracts were also exposed to cultures of both *Salmonella typhimurium* and *Escherichia coli* carrying genetic markers to detect forward and reverse mutation produced by both base-substitution and frameshift DNA changes.

Yeast cells were incorporated with mussel extracts and minimal selective medium in the presence of sufficient growth supplement to enable the cells to undergo three rounds of cell division. *S. typhimurium* was used in a plate incorporation system, as described by Ames *et al.*⁷. *E. coli* was used in both plate incorporation tests and modified bacterial fluctuation tests, as described by Green *et al.*⁸.

Alcoholic extracts were prepared from mussel samples collected from Plymouth (heavy visual pollution), Mumbles, Caswell Bay and Anglesey (clean water area), all in the United Kingdom. The results of exposure of yeast cultures auxotrophic for histidine and tryptophan to mussel extracts at concentrations up to 4% are shown in Table 1. Significant increases in the production of both histidine and tryptophan prototrophs were produced by mussel extracts from Plymouth, Mumbles and Caswell Bay, whereas no such increases were detectable in the presence of mussel extracts derived from the clean waters of Anglesey. Table 1 also contains the positive results of treatment in identical conditions with ethyl methane sulphonate, and the negative result obtained when alcohol was used as a control. Table 1 also shows the results of mussel extracts (Mumbles) derived from either liver tissue alone or non-liver tissue. When prepared in this manner, most of the genetic activity was found to be due to the non-liver extracts.

Table 1 Induction of mitotic gene conversion by mussel extracts

Treatment	Mean no. of <i>his</i> ⁺ prototrophs per plate	<i>his</i> ⁺ prototrophs per 10 ⁶ survivors ± s.e.	Mean no. of <i>trp</i> ⁺ prototrophs per plate	<i>trp</i> ⁺ prototrophs per 10 ⁵ survivors ± s.e.	% Viability
Control 1	8.6	26.8±4.6	7.4	23.1±4.3	91.4
Control 2	7.8	32.5±5.8	5.2	21.7±4.8	68.6
4% Alcohol control 1	6.5	25.6±5.0	9.4	37.0±6.0	73.1
4% Alcohol control 2	4.2	11.8±3.0	7.6	21.4±3.9	101.4
Plymouth extract 4%	202.6	493.6±21.3	154.8	463.5±18.6	95.4
Mumbles extract 4%	71.2	323.6±19.2	85.3	387.7±20.6	62.9
Caswell Bay 4% extract	69.0	276.0±16.6	69.2	276.8±16.6	71.4
Anglesey 4% extract	4.7	18.3±4.2	7.6	29.6±5.4	73.4
Mumbles liver 4% extract	10.8	40.0±6.1	11.4	42.2±6.3	77.1
Mumbles non-liver 4% extract	66.0	307.0±18.9	123.2	573.0±25.8	61.4
Ethyl methane sulphonate (1 µg ml ⁻¹)	284.7	900.9±26.7	346.2	1,095±29.4.7	90.3

Mussel samples were prepared by the extraction of 50 shelled mussels in 100 ml of 95% ethanol in a Waring blender. After centrifugation at 5,000g, extracts were filter sterilised and stored at 4 °C. Stationary phase cultures of the yeast strain *JDI*⁹, auxotrophic for histidine and tryptophan were suspended in saline at a concentration of 10⁷ cells per ml. Samples of mussel extract (up to 4%) were added to yeast minimal medium at 45 °C together with yeast cells at a concentration of 35 × 10³ cells per ml for detection of histidine independent prototrophs and 35 × 10³ cells per ml for detection of tryptophan-independent prototrophs and poured into 9-cm plastic Petri dishes. The culture medium was supplemented with 20 µg ml⁻¹ tryptophan and 0.1 µg of histidine for detection of histidine-independent prototrophs and with histidine (20 µg ml⁻¹) and 0.1 µg of tryptophan for detection of tryptophan independent prototrophs. These supplements enabled auxotrophic cells to undergo three cell divisions in the presence or absence of the mussel extract. All plates were grown in the dark at 28 °C and scored after 9 d of incubation. The possible nutrient effects of the Plymouth mussel extracts on auxotrophic yeast cultures were determined in two ways. (1) After incubation of plates as described above, disks of agar 9 mm in diameter were removed from areas of the selective plates showing background growth, but no large prototrophic colonies. Agar disks sampled from 10 plates per treatment were added to 20 ml of sterile saline and the yeast cells contained in the disks were suspended by sonication. After appropriate dilutions, the saline suspensions were plated on nutrient agar and viable cells were counted after 5 d of incubation at 28 °C. Comparisons of the viable cells contained in the background agar of both treated and untreated cultures indicated that there was no evidence of further growth of auxotrophic yeast cells in solid medium in the presence of mussel extract. (2) Samples of mussel extract were added to liquid minimal medium containing 10⁵ yeast cells per ml and the cultures were aerated at 28 °C for 24 h. Cell growth was determined by counting with a haemocytometer. In no case could we detect increased growth of cells in liquid medium in those cultures containing mussel extracts.

Table 2 Mutation induced in *E. coli* tester strains by MMS and mussel extracts as shown by the fluctuation test

<i>E. coli</i> strain	Locus	Treatment	No. of tubes sampled	Control tubes	Positive test	Significance (probability)
343/113	<i>arg₅₆</i>	MMS	50	21	39	$P < 0.001$
		Mumbles (L)	50	24	34	$P < 0.05$
		Mumbles (NL)	50	24	41	$P < 0.001$
343/113(R46)	<i>arg₅₆</i>	MMS	50	28	48	$P < 0.001$
		Mumbles (L)	50	35	41	NS
		Mumbles (NL)	50	35	45	$P < 0.01$
343/113(R46)	<i>galR₁₈^s</i>	MMS	100	60	99	$P < 0.001$
		Mumbles (L)	100	60	80	$P < 0.001$
		Mumbles (NL)	100	60	95	$P < 0.001$
343/113(R46)	<i>arg₅₆</i>	Burry Estuary (L)	50	13	20	NS
		Burry Estuary (NL)	50	13	31	$P < 0.001$
		MMS	50	22	32	$P < 0.05$
343/113	<i>lys₈₀</i>	Mumbles (L)	50	27	33	NS
		Mumbles (NL)	50	27	29	NS
		MMS	100	48	75	$P < 0.001$
343/113(R46)	<i>lys₈₀</i>	Mumbles (L)	100	65	67	NS
		Mumbles (NL)	100	65	68	NS
		Burry Estuary (L)	50	25	31	NS
343/113(R46)	<i>lys₈₀</i>	Burry Estuary (NL)	50	25	28	NS
		MMS	50	20	33	$P < 0.01$
		Mumbles (L)	50	13	18	NS
WP2 <i>uvrA</i>	<i>trp</i>	Mumbles (NL)	50	13	18	NS
		MMS	100	54	87	$P < 0.001$
		Mumbles (L)	100	48	59	NS
WP2 <i>uvrA</i> (R46)	<i>trp</i>	Mumbles (NL)	100	48	70	$P < 0.005$
		Burry Estuary (L)	100	54	56	NS
		Burry Estuary (NL)	100	54	63	NS

Fluctuation tests were carried out, with minor modifications, as described by Green *et al.*⁸. Overnight cultures of the *E. coli* tester strains were prepared in supplemented Davis-Mingioli minimal medium. After washing twice in saline, the cells were resuspended in saline at a concentration of 5.0×10^7 cells per ml. To 100 ml of Davis-Mingioli basal salts was added 10 ml of 40% glucose (for MMS), 0.1 ml of tryptophan (for liver tissue extracts) solution containing 200 µg per ml (for fluctuation tests involving tryptophan auxotrophs only) or 0.1 ml of lysine (for liver extracts) solution containing 200 µg per ml (for lysine auxotrophs only), 0.1 ml of washed cells, and in the case of the test treatments, either MMS to a final concentration of 1 µg per ml or 0.1 ml of alcohol mussel extract (liver or non-liver). Control experiments were conducted as for the test treatments but omitting MMS or mussel extracts. Controls for experiments involving mussel extracts also contained 0.1 ml of 96% ethanol. Each treatment was dispensed in 2.0-ml samples into 50 small test tubes and incubated at 37 °C. Once the auxotrophic bacteria had exhausted the small amount of supplement present, only prototrophic revertants continued to grow. From 2 d onwards, tubes in which mutation to prototrophy had occurred became turbid, while the other tubes remained relatively clear. The number of turbid tubes was determined routinely after 3 d. *E. coli* strains 343/113 and 343/113 *lys₈₀* were gifts from Dr G. Mohn and *E. coli* WP2 *uvrA* was a gift from Dr M. H. L. Green. To investigate the possible presence of free amino acids in mussel extracts that would increase spontaneous mutation rates by enhancing cell division, viable counts were made from individual cultures of *E. coli uvrA trp* (R46) obtained from fluctuation experiments with and without Mumbles mussel extract. It was found that the median number of bacteria per clear tube, that is tubes that did not contain *trp*⁺ revertants, was unaffected by the presence of mussel extract, and therefore Mumbles mussel extract did not enhance the growth of *trp*⁻ cells when the tryptophan supply has become exhausted. For tests involving the *galR₁₈^s* system, galactose replaced glucose in the fluctuation medium, also as this marker gives a high spontaneous number of turbid tubes each treatment was dispensed in 1.0-ml samples into 100 test tubes. The *arg₅₆* mutation is leaky and as a result some residual growth was observed even in the absence of arginine and therefore trace amounts of arginine were not added when it was used. L, liver, NL, non-liver, NS, not significant.

Table 2 shows the effects of both methyl methane sulphonate (MMS) and mussel liver and non-liver extracts on various strains of *E. coli* as measured by fluctuation tests. *E. coli* 343/113 *arg₅₆*, *nad₁₁₃*, *thi*, *lys₈₀* is a K12 strain developed by Mohn *et al.*¹⁰ for the reliable detection of different types of mutagen, utilising markers in the same genetic background. The *arg₅₆* mutation can be reversed, resulting in arginine prototrophy, by both base-change and frameshift mutagens; *gal R₁₈^s* is a forward mutation and the ability to use galactose as a carbon source can also be restored by both types of mutagen. *lys₈₀* reverts to lysine prototrophy predominantly after action by frameshift mutagens (personal communication from G. Mohn). *E. coli* WP2 *uvrA trp* is an *E. coli* B strain recommended by Green and Muriel¹¹ for base-change mutagen detection. The transferable drug resistance factor R46 has been shown to carry mutator genes that can enhance mutagenesis by both frameshift and base-change mutagens^{12,13}. R⁺ strains were constructed by conjugation using *E. coli* J6-2(R46) as a donor¹⁴.

All strains tested showed significant mutation in the fluctuation test after exposure to MMS. The *arg₅₆*, *gal R₁₈^s* and *trp* mutations all showed increased mutation frequency in the presence of 0.1% Mumbles mussel extract although as with the yeast experiments, in general, the non-liver fraction seemed to carry the major genetic activity. In contrast, the *E. coli* cultures carrying the auxotrophic marker *lys₈₀*, which carries a frameshift mutation, failed to show any increase in mutation frequency in the presence of

mussel extracts.

Table 3 shows the results of plate incorporation tests using both *E. coli* and *S. typhimurium*. Again the *E. coli arg₅₆* and *trp* loci showed significant reversion in R-factor-containing strains after exposure to mussel extracts. The *S. typhimurium his* mutants showed an increase in mutation frequency, induced by mussel extracts, in strain TA98, which produces prototrophic colonies by frameshift mutation, but not in strain TA100, which produces prototrophs by base substitution¹⁵. The failure of the latter strain to detect mutations induced by mussel extract reflects the specificity of the strains used.

Control experiments with both bacteria and yeast (briefly outlined in Tables 1 and 2), which will be described in further detail elsewhere, showed that the mussel alcohol extracts did not contain additional nutrient supplementation. Therefore the increases in prototrophs observed with the various strains were due to induction and not to a simple increase in cell division with a concomitant increase in spontaneous mutation. Further controls also showed that the mussel extracts were free of radioactive material.

Our results provide convincing evidence for the presence of mutagens that have become concentrated in the body tissues of mussels obtained from a number of maritime environments. The numerous observed correlations between chemicals showing convertogenic or mutagenic activity and carcinogenic activity¹⁵⁻¹⁷, suggest that such agents also represent a possible source of chemically induced carcinogenesis. At present our results do not provide direct

Table 3 Mutation induced in *E. coli* and *S. typhimurium* tester strains by mussel extracts as shown by plate incorporation tests

Strain	R factor	Mussel extract	Locus	Mean no. of prototrophs per plate
<i>E. coli</i> 343/113	—	Control	<i>arg</i> ₅₆	4.0 ± 1.0
		Mumbles (L)		9.0 ± 1.8
		Mumbles (NL)		9.9 ± 1.5
<i>E. coli</i> 343/113	+	Control	<i>arg</i> ₅₆	206.3 ± 11.3
		Mumbles (L)		671.0 ± 21.0
		Mumbles (NL)		Not done
<i>E. coli</i> WP2 <i>uvrA</i>	—	Control	<i>trp</i>	4.0 ± 1.0
		Mumbles (L)		6.3 ± 1.2
		Mumbles (NL)		7.0 ± 1.2
<i>E. coli</i> WP2 <i>uvrA</i>	+	Control	<i>trp</i>	28.3 ± 3.6
		Mumbles (L)		38.7 ± 6.2
		Mumbles (NL)		72.7 ± 3.5
<i>S. typhimurium</i> TA98	+	Control	<i>hisD</i> ₃₀₅₂	13.6 ± 1.6
		Plymouth (W)		104.5 ± 4.6
		Control	<i>hisG</i> ₄₆	61.5 ± 5.5
<i>S. typhimurium</i> TA100	+	Plymouth (W)		74.0 ± 6.1
		Control		5.5 ± 1.0
<i>S. typhimurium</i> TA98	+	Mumbles	<i>hisD</i> ₃₀₅₂	22.0 ± 2.1
		Control		163.8 ± 5.7
		Mumbles		174.0 ± 5.9

The method used was essentially that of Ames *et al.*⁷. Overnight cultures of the tester strains were washed in saline and resuspended in saline. Washed bacterial culture (0.1 ml) plus 0.1 ml of mussel extract were added to semimolten minimal agar at 46 °C containing glucose and relevant supplements. These overlays were poured over minimal media plates containing glucose. The plates were normally scored after 3 d of incubation at 37 °C. W, whole mussel extract; L, liver; NL, non-liver.

evidence of the nature of the chemicals we detected. But the active extracts came from mussels collected in areas with obvious industrial pollution or in close proximity to industrial areas.

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Species richness and stability of space-limited communities

ECOLOGICAL competition theory has reached its fullest state of development for the special case of competition for a renewable resource^{1,2}. There are, however, many instances of competition in nature to which this theory does not apply; for example, competition for space (*Lebensraum*). Because there are many space-limited organisms in nature, including many primary producers, this is an important gap in our theoretical under-

standing of ecological communities. Moreover, since the fundamental dynamic of competition for space—spatial mobility—is quite different from that of competition for a renewable resource, it would be unwise to try to extrapolate the theory of resource limitation to space-limited communities. In this paper, I outline some calculations, in the spirit of refs 3–7, which bear on the relation between stability and diversity for space-limited communities. (Full details will appear elsewhere.)

It is now widely accepted that in general mathematical models of multispecies communities, complexity *per se* tends to beget instability rather than stability (ref. 2, ch. 3). There are also traces of this tendency in space-limited communities, but for a certain class of space-limited communities, complexity (in the sense of species richness) promotes (local) stability.

The model used is a system of reaction-diffusion equations^{8,9} of the form

$$\frac{dx_i^\mu}{dt} = \left[C_i x_i^\mu \left(k_i - \sum_j a_{ij} x_j^\mu \right) \right] + \left[D \sum_v (x_i^v - x_i^\mu) \right] \quad (1)$$

The meaning of this equation is as follows (for a more detailed description, see ref. 6). The habitat is partitioned into m patches of space and is populated by n species; x_i^μ , $\mu \in \{1, \dots, m\}$, $i \in \{1, \dots, n\}$, is the number (or biomass) density of species i in patch μ . Within each patch, the species compete among one another according to (for the sake of concreteness) a Lotka–Volterra model. This is expressed by the first square bracket in equation (1), in which k_i is the carrying capacity for species i , the a_{ij} are the competition coefficients, and $C_i k_i$ is the growth rate of species i . The competition coefficients a_{ij} are chosen so that the corresponding Lotka–Volterra model allows no stable equilibrium with any pair of species present (which seems the most characteristic sort of competition for space). Each species is, however, allowed to disperse randomly among the patches at a rate characterised by a constant D ; this dispersion is such that there will be a net flow of a given species from patches with a relatively high density into patches with a relatively low density of that species. This is expressed by the second half of equation (1).

Consider colonisation of newly available space in such a model, taking the following initial conditions: the n species are randomly distributed among the m patches, with only 1 species per patch, and its density is in each case near its carrying capacity. Using the above equations the system will, for sufficiently small D , approach a stable equilibrium, with all species present in each patch⁶. I will call this a ‘colonisation equilibrium’ of the model.

The stability behaviour of an equilibrium can be characterised by means of the quantity $\Lambda = \text{minimum } \{-\text{Re}(\lambda_A)\}$, where $\{\lambda_A: A = 1, \dots, mn\}$ are the eigenvalues of the linearised stability matrix of the system, evaluated at the equilibrium². ($\text{Re}(z)$ means the real part of z , for any complex z .) The condition for stability in the sense of Liapunov is $\Lambda > 0$ (which is just the familiar condition that all the eigenvalues have negative real parts), but the magnitude of Λ contains additional information: in case the equilibrium is Liapunov stable, then Λ^{-1} is of the order of the time scale for the exponential return of the system to equilibrium, if it has been moved away from equilibrium by some (small) disturbance. Alternatively, if the effects of random environmental fluctuations are represented by taking some parameter in the model to be a Gaussian random variable with variance σ^2 , then an approximate criterion for ‘stochastic stability’ of the equilibrium (meaning that the environmental fluctuations are unable to drive the system very far from equilibrium) is $\Lambda > \sigma^2$ (refs 2, 10). Thus, among Liapunov stable equilibria, Λ is a meaningful measure of the degree of stability: the larger Λ , the more stable the equilibrium.

To determine how the stability of colonisation equilibria of the above family of models depends on the number, n , of colonising species systems with different values of n are needed

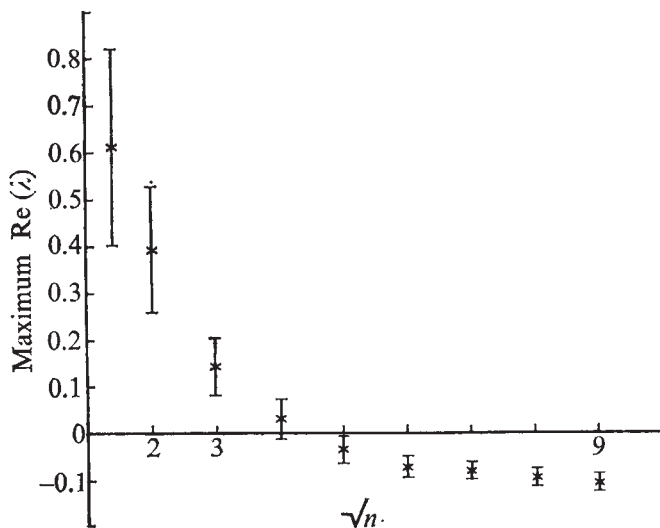


Fig. 1 The average (for the statistical ensemble defined in the text) largest real part of the stability matrix eigenvalues against $\sqrt{n}$, calculated by a numerical Monte Carlo method, for the parameter values $m = 1,000$, $D = 0.0002$, $(a, b) = (1.1, 1.9)$. The error bars are for one standard deviation. For systems chosen entirely at random, this quantity increases with n , rather than decreasing as here (ref. 2, Fig. 3.6).

which are, however, comparable in an appropriate sense. First, for a fixed habitat, the number, m , of patches is fixed throughout the following discussion. For each value of n , consider the statistical ensemble of systems having this number of species, for which the various parameters of the system are chosen at random from some fixed intervals of real numbers, and the n species are initially distributed at random among the m patches, with one species per patch. The average value of Λ is calculated for each n for the ensuing colonisation equilibria over this ensemble, and is $\langle \Lambda(n) \rangle$. The function $\langle \Lambda(n) \rangle$ of n indicates how, on average, the stability of such systems varies with n .

It transpires that there are two possibilities. Let (a, b) be the interval from which the competition coefficients α_{ij} ($i \neq j$) are chosen. Normalising α_{ii} to one for all i and scaling the species densities so that the carrying capacities k_i are all equal to unity, the condition for instability of all pairwise interactions (without dispersion) is $\alpha_{ij} > 1$ for all i, j with $i \neq j$. (This assumes no functional dominance, which will be discussed elsewhere in these pairwise interactions.) $1 < a < b$ must always be the case. The choices made for the other parameter intervals do not affect the qualitative nature of the outcome, which is as follows. If $a > 2$, then $\langle \Lambda(n) \rangle$ is a monotonically decreasing function of n , and if $(a+b)/2 < 2$, then $\langle \Lambda(n) \rangle$ is a monotonically increasing function of n .

Figure 1 shows an example of this latter behaviour (with $(a, b) = (1.1, 1.9)$). To facilitate comparison with earlier results² for stability matrices chosen completely at random, I have plotted the average largest real part of the stability matrix eigenvalues (that is, $-\langle \Lambda(n) \rangle$) against $\sqrt{n}$. The error bars are for one standard deviation. Figure 1 compares directly with Fig. 3.6 of ref. 2: the numbers depicted were obtained by a Monte Carlo method.

So, for $a > 2$ (which means roughly that the competition is constrained to be sufficiently "strong") increased species richness tends to destabilise the colonisation equilibria, whereas for $(a+b)/2 < 2$ ("weak" competition) increased species richness tends to stabilise the colonisation equilibria. The implications of this for the overall stability of the system depends on the global structure of each model. For some models, stochastic instability of the colonisation equilibria, in the sense that the variance σ^2 of environmental fluctuations exceeds Λ , will mean that the spatial structure of the system will be fluctuating constantly from one pattern to another, but with all n species present (compare ref. 7); whereas for other models it will mean

a high probability that one or more species will become extinct, on an ecological time scale.

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Effects of drought on grain growth

DROUGHT severely decreases grain yield in many cereal growing regions and the resulting fluctuations in world food supply have serious repercussions¹. Crop breeders and physiologists are therefore studying varietal characteristics that may enable cereals to yield more consistently in drought conditions². Very few precise field measurements show how drought affects grain growth, but procedures have been advocated to provide a more precise description of grain growth and a more rigorous examination of sources of assimilate used for grain growth³⁻⁵. We have used these procedures to analyse how the droughts of 1975 and 1976 affected grain growth of wheat crops in England and to define particular physiological traits which govern yield.

The analysis is based on detailed measurements of crop growth made on three crops of Maris Huntsman winter wheat, grown in the Midlands on the same soil type in 3 consecutive years. The crops were grown according to normal agronomic practice and were chemically protected against diseases, pests and weeds. Actual evaporation (E_a) was measured using a neutron probe technique⁶ and potential evaporation (E_p) was estimated from a standard formula⁷. Table 1 gives the grain yields of the three crops and pertinent weather observations: 1976 was the driest growing season at Sutton Bonington since records began in 1916. E_p and temperature during grain growth increased markedly from 1974 to 1976, whereas E_a decreased.

For analysis, grain growth rate can be assumed constant^{3,8,9} and final mean weight per grain ($\bar{W}_g(f)$) can be expressed as:

$$\bar{W}_g(f) = \bar{W}_g(i) + (R_g \times D_g) \quad (1)$$

where $\bar{W}_g(i)$ is the initial value of $\bar{W}_g$, R_g is the grain growth rate during the linear phase of growth and D_g is the duration of that phase³. Increase in $\bar{W}_g$ was assumed to be linear once $\bar{W}_g$ exceeded 6 mg, and Fig. 1 shows the grain growth pattern

Fig. 1 Increase in mean weight per grain with time in 1974 (□), 1975 (△) and 1976 (○).

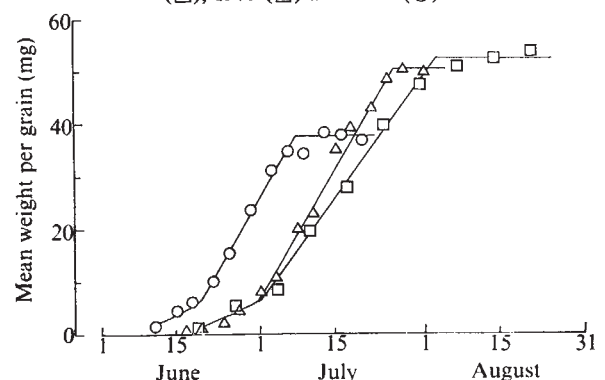


Table 1 Grain yield, weather and grain growth observations for the three wheat crops

Year	Dry matter grain yield* (g m ⁻²)	Duration (d)	Anthesis until cessation of grain growth			During linear grain growth				Final mean weight per grain (mg)
			Actual evaporation [E _a] (mm d ⁻¹)	Potential evaporation [E _p] (mm d ⁻¹)	E _a /E _p	Mean air temperature (°C)	Mean maximum air temperature (°C)	Mean grain growth rate [R _g] (mg d ⁻¹)	Grain growth duration [D _g] (d)	
1974	495 (46)	45	3.0	3.0	1.00	14.9	19.5	1.41	32.9	52.2
1975	535 (51)	38	2.0	3.4	0.59	16.8	21.6	1.76	25.2	50.3
1976	340 (32)	25	2.1	4.6	0.46	20.7	28.5	2.11	14.5	36.6

*Farm yields in cwt acre⁻¹ at 15% moisture are shown in parentheses.

for the 3 yr. Table 1 gives the relevant values of the variables of equation (1). Compared with 1974 the warmer, drier weather of 1975 was associated with a slightly faster R_g but this was offset by a decrease in D_g so that $\bar{W}_g(f)$ was close to 50 mg in both seasons, a typical figure for Maris Huntsman¹⁰. In 1976 very dry weather and exceptionally high temperatures were associated with very fast R_g (Table 1). But this was more than offset by a sharp decrease in D_g so that $\bar{W}_g(f)$ was only 37 mg.

Changes of plant dry weight after anthesis must be examined in greater detail to establish the reason for the extremely short D_g in 1976.

It is convenient to distinguish two sources of assimilate supply for grain growth: photosynthesis after anthesis, and translocation of materials assimilated before anthesis and temporarily stored before moving to the grain. As a first step in assessing the importance of these two sources, increase in grain weight from anthesis to final harvest (ΔW_g) can be expressed as

$$\Delta W_g = \Delta W_t - \Delta W_s$$

where ΔW_t is the increase in total crop weight, and ΔW_s is the increase in weight of plant parts other than the grain between anthesis and harvest⁵. By far the largest proportion of ΔW_s consists of the stem and for brevity this term is referred to as stem weight. In Table 2, which shows the relevant crop dry weight changes, $-\Delta W_s/\Delta W_g$ is the fraction of final grain weight apparently derived from translocation of materials assimilated before anthesis. Even in 1974, this fraction (0.35) was much larger than the maximum of about 0.20 expected for wheat¹¹. In 1975 and 1976, however, when production of dry matter after anthesis was smaller still in relation to the requirements for grain growth, the contribution of translocated materials to grain yield was much greater. This increased movement of materials from stem to grain when conditions during grain filling are adverse for photosynthesis lends support to the concept of "compensatory translocation"⁵. The small value of $\bar{W}_g(f)$ which occurred in 1976 in spite of considerable translocation of material from the stem raises two questions: is there a limit to the amount of dry matter that can be supplied to the grain by compensatory translocation; was this limit reached or exceeded in 1976?

The extent of translocation may ultimately be limited by the need to retain sufficient dry matter in the stems to support the ears. In 1976, however, supporting tissue per grain was considerably greater than in 1975 (Table 2). Thus it seems that shortage of assimilate did not limit final grain weight by curtailing the duration of grain growth. There are two other possible reasons why grain growth stopped: either the transport

system to the growing grain was inadequate or starch synthesis stopped prematurely. Studies of translocation during wheat grain growth show little effect of water stress¹², but there is evidence that a fall in the synthetic capacity of the endosperm causes grain growth to stop¹³. In 1975 and 1976, the duration of starch synthesis could have been limited either by the effect of drought on plant water status or by abnormally high ear temperatures. The relative importance of these two correlated factors cannot be determined from our measurements, but related work in controlled environments indicates that temperature rather than water stress stops grain growth prematurely¹⁴⁻¹⁶.

These results illustrate the potential importance of translocation of material assimilated before anthesis to grain growth when current photosynthesis is limited by an adverse environment. They also establish the usefulness of treating grain growth as a linear process and expressing final weight per grain as a product of a growth rate and duration. If cereals are to yield well in regions where drought and high temperatures during grain filling are frequent, varieties must be bred which not only increase the rate but also sustain the duration of grain growth. Such characteristics would maximise the assimilate to be harvested in the nutritionally valuable fraction of the crop.

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Table 2 Crop weight measurements

Year	Increase in weight (gm ⁻²) between anthesis and harvest				Stem weight per grain (mg)
	Grain [ΔW_g]	Total [ΔW_t]	Stem [ΔW_s]	$-\Delta W_s/\Delta W_g$	
1974	495	322	-173	0.35	65
1975	532	228	-304	0.57	54
1976	341	149	-192	0.56	63

Predicting the course of Gompertzian growth

THE increase in volume or size with time that characterises many biological and physical systems is often well approximated retrospectively by mathematical 'growth curves'. In some cases, however, growth may be sufficiently complicated for it to be impossible to predict later portions of the growth curve if observations are limited to a few early points. We report here the development of a generalised approach to

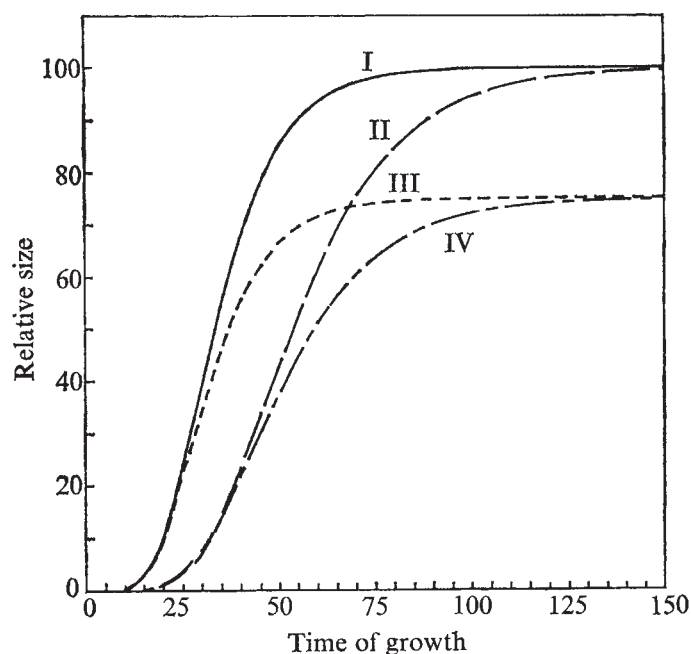


Fig. 1 Hypothetical Gompertzian curves derived by solving equation (3) with various parameters A and B as follows: Curve I, $A = 0.4$, $B = 0.4/(\ln 100)$; Curve II, $A = 0.25$, $B = 0.25/(\ln 100)$; Curve III, $A = 0.4$, $B = 0.4/(\ln 75)$; Curve IV, $A = 0.25$, $B = 0.25/(\ln 75)$. Note that the early portion of the curve reflects parameter A and the later portion of the curve reflects the ratio of A/B .

the analysis of "Gompertzian" growth which enables accurate predictions of future growth for two model tumour systems. This mathematical method may be useful clinically, and expresses a property of biological growth that may be applicable to other systems.

The growth of most experimental neoplasms²⁻⁴, human populations⁵, normal embryos⁶, molluscs⁷, visceral organs^{8,9} and some human tumours¹⁰ are well described by the S-shaped curves used for actuarial purposes by Benjamin Gompertz in 1825 (ref. 1). The essential distinguishing feature of Gompertzian growth (Fig. 1), is some mechanism of feedback inhibition with increasing size, resulting in decremented exponential growth that achieves a limiting size asymptotically. Unfortunately, the relatively complicated form of the Gompertzian equation has tended to limit its general applicability, often resulting in the alternative use of less satisfactory exponential¹¹⁻¹⁴, summed exponential¹⁵, or other¹⁶ growth models. These other models however, are rarely applicable to an entire growth history, and are therefore insufficient for the purposes of predicting later growth.

A Gompertzian function is the simultaneous solution of the two differential equations

$$dN(t)/dt = K_1 \cdot N(t) \cdot G(t) \quad (1)$$

$$dG(t)/dt = -K_2 \cdot G(t) \quad (2)$$

where K_i is a constant > 0 , $N(t)$ is volume at time t , and $G(t)$ is a function entirely described by equation (2). The expression $K_2 \cdot G(t)$ can be thought of as the fraction of $N(t)$ that doubles in size during the instant dt . The solution of these equations is often written in the form

$$N(t) = N(0) \cdot \exp[(K_1/K_2) \cdot G(0) \cdot \{1 - \exp(-K_2 \cdot t)\}]$$

Since

$$G(t) = G(0) \cdot \exp(-K_2 \cdot t) = (K_2/K_1) \cdot \ln[N(\infty)/N(t)]$$

where $N(\infty) = \lim_{t \rightarrow \infty} N(t)$, an alternative form of equation (1) is

$$\begin{aligned} dN(t)/dt &= K_2 \cdot N(t) \cdot \ln[N(\infty)/N(t)] \\ &= A \cdot N(t) - B \cdot N(t) \cdot \ln[N(t)] \end{aligned} \quad (3)$$

where $B = K_2$ and $A = K_2 \cdot \ln[N(\infty)]$. The dependence of the pattern of growth of $N(t)$ on A and B is illustrated in Fig. 1. For a given $N(0)$, when A is constant, the early phase of growth (often referred to loosely as the exponential phase) is similar in spite of different values of B . Also, when A/B is constant, the later phase of growth (the plateau phase) is similar in spite of different values of A . Were A and B unrelated, knowledge of the early phase of growth of a particular system (tumour, organ, embryo, or population) would not enable an accurate prediction of the later phase, as the value of A , but not A/B , could be estimated from the early growth pattern. Similarly, measurements of late growth would provide an estimate of A/B but not A . On the other hand, if it were possible to estimate B from A , the entire growth pattern of an individual system would be predictable on the basis of measurements early in its growth history.

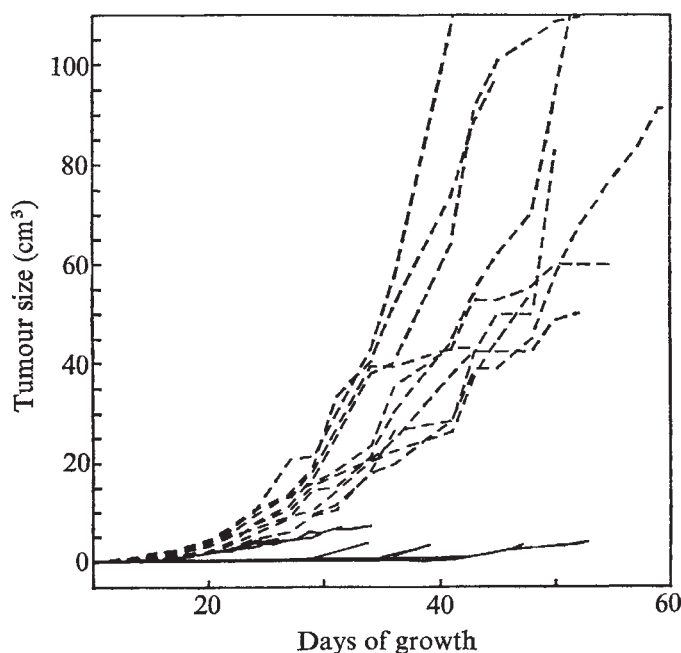
For eight cases of B-16 melanoma in BDF₁ mice and ten cases of transplantable mammary carcinoma 13672 in Fischer 344 rats, measurements of length (L) and width (W) were obtained during unperturbed growth from the time of transplant until the death of the host or the occurrence of tumour ulceration. Approximate tumour volumes (V) were calculated as the volume of revolution of an ellipse by the formula

$$V = (\pi/6) L W^2$$

The growth of these tumours (Fig. 2) demonstrates the variability typical of malignant proliferation.

For each tumour, the solution of equation (3) was fit using the method of least squares to the curve of volume as a function of time of growth starting from the hypothetical volume of a single cell. Examples of curves fitted to data for each tumour type are illustrated in Fig. 3. Each curve fit yields the two parameters, A and B , that completely describe the growth pattern

Fig. 2 The volume of eight examples of B-16 melanoma (—) and ten examples of 13672 carcinoma (---) plotted as a function of time from initiation of growth. The volume scale is arithmetic. The variability in growth pattern typical of neoplastic proliferation is evident.



for that tumour. As seen in Fig. 4, when $\exp(A)$ is plotted against $\ln(B)$ for all individuals of each tumour type, the relationship is found to be linear with correlation coefficients of 0.99 for B-16 melanoma ($P < 0.0000002$) and 0.96 for the rat carcinoma ($P < 0.000006$). This high degree of correlation between the transforms of A and B allows, for these experimental tumours, the accurate prediction of B on the basis of an estimate of A made from measurement of the early phase of growth of an individual tumour. Equation (3) may therefore be written

$$dN(t)/dt = A \cdot N(t) - \exp\{[\exp\{A\} - n]/m\} \cdot N(t) \cdot \ln[N(t)] \quad (4)$$

a Gompertzian equation in one variable, A , with constants n , m , and $N(0)$ (the volume of a single cell) specific for a particular tumour type.

Having determined empirically the constants n and m for a tumour type, the total growth curve for an individual tumour of that type can be predicted accurately from a few early measurements of volume. In Fig. 3, for example, the solution of equation (4) was fit to three early measurements of a tumour not used to derive the above relationship between A and B . The prediction obtained is seen to match closely the actual volumes observed later in the growth of the tumour.

The ability to predict the later growth pattern of an individual tumour on the basis of early growth measurements should have widespread applicability, not only in the design of experimental anti-cancer therapy, but in the diagnosis and prognosis of human tumours as well. For example, the response of an individual cancer to treatment could be expressed in terms of deviation from ideal unperturbed growth as predicted from measurements taken before therapy. This precise method of monitoring therapy has obvious advantages over parameters presently in use, such as rate of complete or partial remission or average tumour size at some arbitrary point after therapy. These conventional endpoints are inapplicable to individual

Fig. 3 Examples of equation (3) fit to data for B-16 melanoma ($\square$) and 13762 rat carcinoma (Δ). The volume scale is logarithmic. The dashed lines represent the fitted curves. Paired constants (A , B) derived from several such curve fits are plotted in Fig. 4. The points marked 0 are three early volume measurements for an individual 13762 rat carcinoma not used to derive the constants n and m (see text). Equation (4) was fit to these three points using constants n and m from Fig. 4; the resulting solid line is seen to predict accurately the later growth measurements ($\bullet$) for that tumour; measurements unknown at the time of curve fitting.

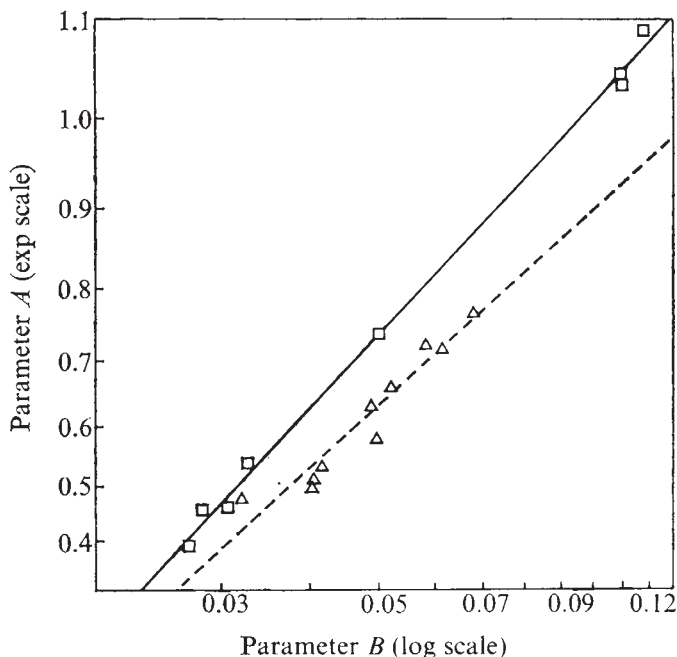
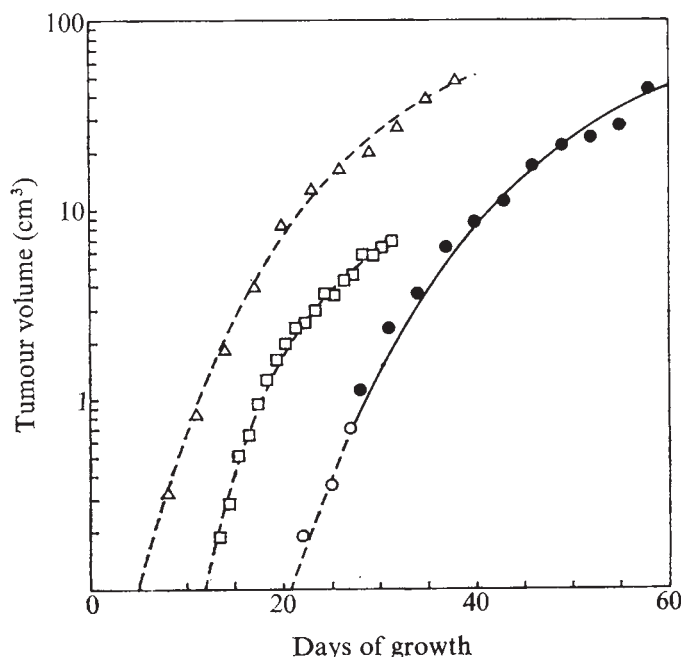


Fig. 4 Correlation between $\exp(A)$ and $\ln(B)$ for B-16 melanoma ($\square$) and 13762 rat carcinoma (Δ). The points represent pairs of parameters (A , B) determined from curve fits as illustrated in Fig. 3; the solid lines represent the regression equation $\exp(A) = m \ln(B) + n$, with the following values of m , n , respectively: B-16 melanoma, 0.9645 and 4.980; 13762 carcinoma, 0.8176 and 4.334.

tumours, are potentially biased by drug or other non-tumour deaths, and may underestimate the influence of weakly effective forms of therapy which may nevertheless be potentially useful in combinations.

For these two experimental tumours the methods described here reveal a remarkable orderliness of growth that tends to dispel the concept of cancer as a wild and uncontrolled phenomenon. We may expect that the growth of non-neoplastic tissues (for example, gut epithelium, bone marrow and regenerating liver) would be at least as orderly as that of these two malignancies. Results obtained by applying these methods to normal tissues will be reported later. These mathematical methods may also be applied to the growth of mammalian or bacterial cells in culture, with obvious use in the monitoring of growth inhibitory influences (such as hormones and antibiotics). We also plan to describe the applications of these methods to other tumour types, paying particular attention to the description and prediction of the response of malignancies to single and multiple agent therapies.

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Significance of cell shape in tissue architecture

STIMULI passing to a cell within solid tissue are modulated by the cellular environment. Control mechanisms must therefore involve both humoral influences and the architecture of the cellular microenvironment. Epidermis suffers a constant loss of surface cells which are replaced by basal cells which have differentiated during migration to the surface. In mouse ear and dorsum, the path of this migration can be observed as precisely defined columns of cells¹ which are in tetrakaidecahedral² form (Fig. 1) as in the rigid cell walls of plant tissues³. Recent studies^{3,4} have suggested that maintenance of this system of 'dynamic orderedness' may be explained by reference to its physical geometry, and minimal surface packing configurations. In this paper we discuss some of the consequences of this view of tissue architecture.

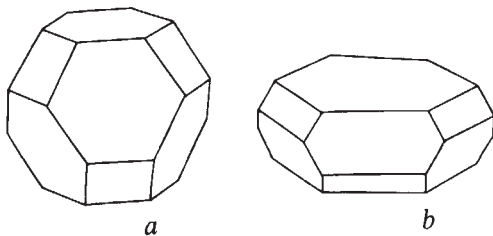


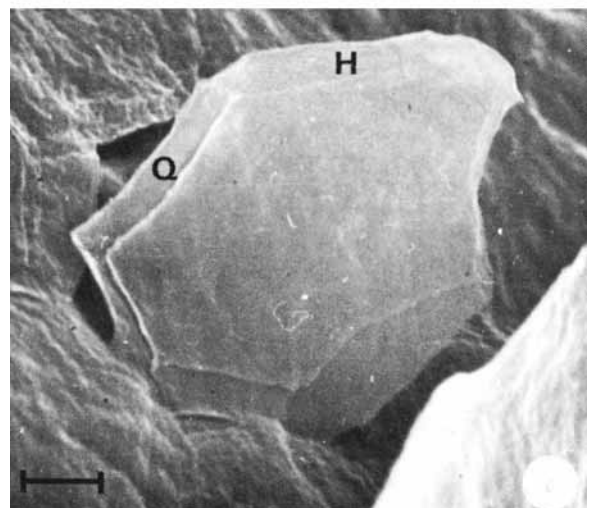
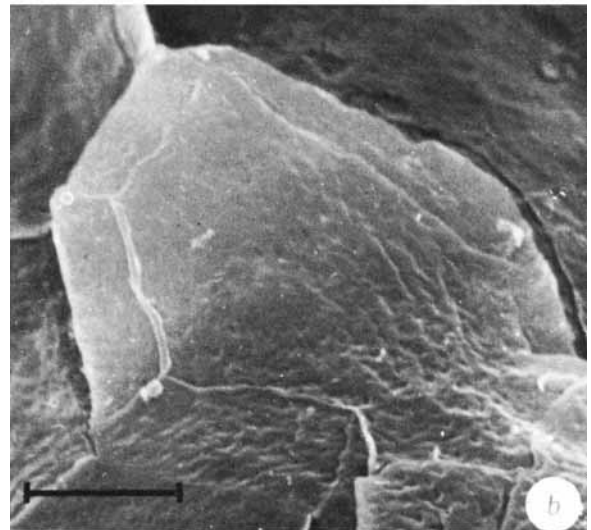
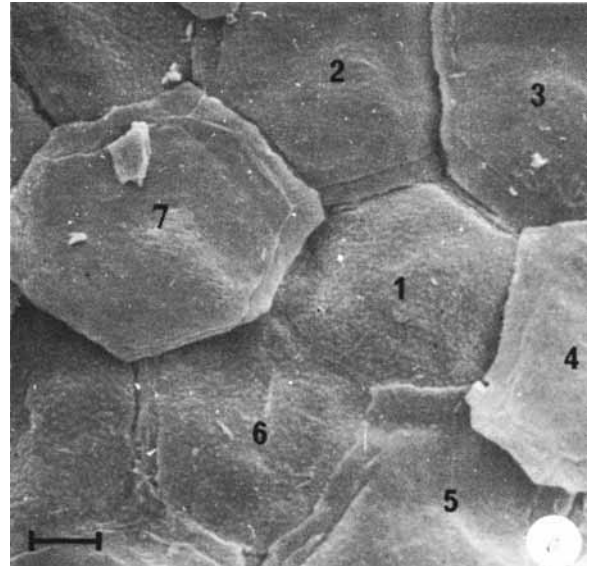
Fig. 1 *a*, Line drawing of an orthic tetrakaidecahedron. This 14-sided figure consists of six equal opposite quadrilateral faces and eight equal and opposite hexagons. Each quadrilateral face is bounded by four hexagons and each hexagonal face by three hexagons and three quadrilaterals. *b*, When compressed about a pair of hexagonal faces the shape assumed will become predominantly hexagonal, with the remaining 12 faces becoming hexagonal and quadrilateral facets around the edges.

The ordered epidermis of mouse ear or dorsum shows keratinised squames piled in columns and presenting a characteristic hexagonal patterning^{1,5}, when viewed from the surface (Fig. 2). It is clear from the flattened nature of these cells that if they are tetrakaidecahedral, then the orthic form of the tetrakaidecahedron has become distorted to a flattened version (Fig. 1), in which the flattening has resulted in an increase in area of a pair of the hexagonal faces, reducing the remaining 12 faces to alternate quadrilateral and hexagonal facets around the edges.

Fig. 2 *a*, Area of mouse ear epidermis showing a central hexagonal squame (1) and its six surrounding neighbours (2-7). Squame 1 is overlapped on all edges, and so cannot desquamate until all the surrounding cells (2-7) have done so, whereas squame 7 has all edges free and is able to desquamate. The region of overlap (previously covered by the last squame in the central pile) and the interdigitating facets are visible at the edges of the surrounding squames. At this advanced stage of flattening that occurs close to the surface, the facets at the squame edge have largely lost their characteristic alternate hexagonal and quadrilateral appearance because of the overall distortion, but still provide evidence of the tetrakaidecahedral form adopted in the less keratinised and more plastic regions of the granular layer. Scale bar: 5 μ m. *b, c*, Cells exposed from some distance below the surface showing clearly the facets at the edge providing the interdigitating regions. In some cells (*c*) a close approximation to the alternate hexagon (H) and quadrilateral (Q) faceting seen in the true geometric tetrakaidecahedron (Fig. 1*b*) is apparent. Scale bars: 5 μ m.

These facets may be observed in the scanning electron microscope particularly if some of the outer squames are removed (Fig. 2).

From the density packing configuration of either orthic or flattened tetrakaidecahedra, there is only one way in which these shapes can be assembled. This results in a columnar organisation in which the top 'cell' of the central column, of any group selected, occupies a position at the lowest or highest level, relative to the surrounding six adjacent columns which are



themselves subdivided alternately into two sets of three, each set at a different level (Fig. 3) rather than any of the other possible arrangements for hexagonal grid systems^{7,8}. Any individual surface cell may thus be considered to be surrounded by two sets of three, and as the individual itself is also part of a set of three, an entire area is thus split into sets of 'threes'. This approximates the observed structure of mammalian epidermis^{1,3,5,6} and was also suggested from a two-dimensional hexagonal grid system⁷ after consideration of control mechanisms.

Epidermal columns can be defined down to the spinous layer (Fig. 4) (that is the layer immediately above the basal layer) and each column must be maintained by vertical migration (and its associated cell division) in the group of 10 basal cells directly beneath the column as defined by the epidermal proliferative unit (EPU) concept^{5,6}. The cell kinetics of the epidermis⁹ suggest an entry of one cell per day into each column, which must equal the cell loss rate. Cell loss in a tetrakaidecahedral system is limited to individual cells, as each cell only becomes free to desquamate when it is slightly 'proud' of its six neighbours and has all its edges free (position 7 in Fig. 2a). Such an ordered desquamation in relation to tetrakaidecahedral cell shape implies a regular alternating overlap between neighbouring columns, as observed in epidermal sections^{6,7,10}. Communication between neighbouring columns seems necessary to ensure the correct sequence of cell migration⁷. Considered in profile alone (Fig. 4), for the regular alternating overlap of squame edges to be maintained, a column cannot introduce another differentiated cell into its squame pile until both neighbours have added to theirs. Extension of this concept to three dimensions involves consideration of the under surface of a group of seven tetrakaidecahedral columns (Fig. 3, inverted) and shows that the central column must be added to before those at the periphery. The overlaps of the peripheral columns may form a vertical channel to aid this sequence, and thus provide a partial self-assembly system which predetermines the column entered by the next migrating basal cell. This determination of the eventual position of each vertical migration imposes a sequence for cell migration and production on the basal layer. As there is a group of approximately ten basal cells positioned beneath each column (Fig. 4), it seems reasonable to assume that they provide cells directly into the column, as suggested by the EPU concept⁵: migration into a column from outside this group would be unnecessary and

Fig. 3 Drawing of a model of flattened tetrakaidecahedra assembled without interstices showing how the columnar arrangement develops. Three different levels are apparent in the group which consists of a central column (upper surface of the top cell black) and its surrounding six neighbours. The central column here is in the lowest position (black), the three columns arrowed right in the intermediate position, and the three arrowed left in the uppermost position. Thus when the diagram is inverted, the column ready to receive the next migrating basal cell will be the central column, and the raised edges of the six surrounding columns may provide a physical guide to facilitate such a migration.

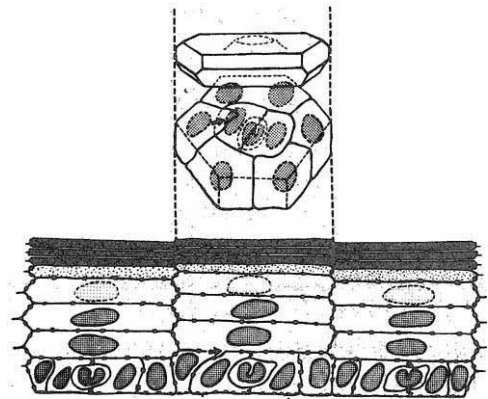
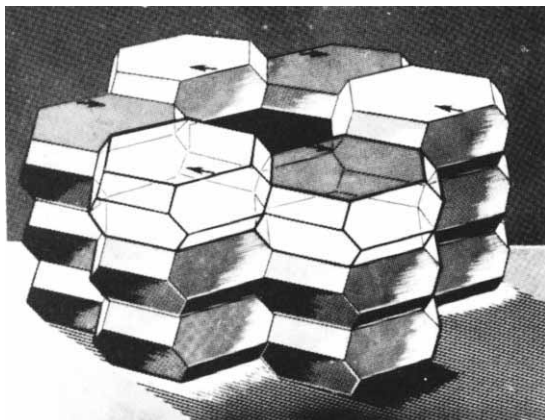


Fig. 4 Diagrammatic representation of three adjacent columns and their underlying basal cells as seen in profile. Here the peripheral columns have been the most recent to add to their stack of differentiating cells so will not add further cells until the central column has added a cell, otherwise the exact overlapping of squame edges would not be maintained. No column or EPU is independent of its neighbours as regards the vertical migration of basal cells into the spinous layer.

inefficient, involving extensive lateral migration of basal cells. A problem thus arises with the direct comparison made by Menton^{3,4} between cell columns in epidermis and columns of soap bubbles. The epidermis is a compressed series of cell layers without intercellular spaces, tightly bound together by desmosomes. Migration from the basal layer involves a major change of cell shape and extensive breaking/remaking of the desmosomal contacts. In a bubble tank, however, there is free space for lateral movement, and no bubble-to-bubble connections, so any bubble may jostle its way to the correct position in a column base regardless of its point of origin. The basic difference is, therefore, that soap bubble columns exist as passively formed stable arrangements of randomly generated bubbles, in free space, whereas skin columns are produced by what can only be an active control of cellular dynamics.

Although the bubble analogy is useful in understanding the precise geometry of the system, it seems unlikely that living cells become formed into columns because they have no option other than to pack in a minimum surface configuration. It is more likely that selection in epidermis has produced the most efficient system of barrier production, which in stacked areas of epidermis assumes a minimum surface configuration. We may also mention at this point that in plantar mouse epidermis, there is no apparent order and also that the orderedness in stacked epidermis breaks down in response to wounding. In stacked epidermis it therefore seems reasonable to view each column as an individually controlled entity, with cell replacement occurring in the group of basal cells directly beneath the column, that is, an epidermal proliferative unit^{5,6}. Two levels of control of the EPU would be necessary to maintain columnar organisation: an intra-EPU control of cell division as a replacement process subsequent to migration, and an inter-EPU control providing a synchrony of vertical migration in trios over the epidermal surface. It seems clear therefore that the ordered structure of columnar epidermis itself precludes random basal cell migration and mitosis, and suggests perhaps that other cell renewal systems with slightly less obvious cellular architecture may maintain themselves by similar systems.

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Transformation of tissue-cultured xeroderma pigmentosum fibroblasts by treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine

In vitro transformation by chemical carcinogens has been shown and established mainly in rodent cells¹. Attempts made to transform tissue-cultured human cells have so far failed². Only two seemingly promising reports on *in vitro* transformation of human cultured cells were those reported by Benedict *et al.*³ and Rhim *et al.*⁴. But the cell line used by Rhim *et al.* to transform by treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) was derived from osteosarcoma and had itself many cytological as well as chromosomal features characteristic of malignant tumour cells. As we cannot imagine why human cells should not be transformed by the chemicals which are potent carcinogens for cells of rodent origin, we believe that successful transformation depends only on finding the optimal experimental conditions and suitable cells.

Xeroderma pigmentosum (Xp) is a recessive hereditary disease characterised by a marked sensitivity to sunlight (ultraviolet irradiation) which often results in formation of skin tumours. This disease is due to a genetic defect of the excision repair system^{5,6}, analogous to the *uvr* mutations in *Escherichia coli*. We have been interested in this disease because it may help to clarify the mechanism of carcinogenesis by allowing the study of the relationship between the excision repair defect and malignant tumour formation. Here we report our preliminary results on an apparent transformation of fibroblasts derived from the skin of two Xp patients by treatment with MNNG.

A human Xp cell line designated CRL1200 was obtained from the American Type Culture Collection and used at 11th subculture level. This cell line was derived from an 11-yr-old girl with neurological complications and skin tumours in addition to ordinary skin symptoms characteristic of Xp; it had a normal fibroblast morphology. Another Xp cell line designated Xp-T was our isolate from a healthy part of skin derived from a 32-yr-old Japanese male with lip cancer in addition to ordinary clinical symptoms of Xp; it was used at its 9th subculture level. As a normal control a fibroblast culture derived from a 26-yr-old Japanese male without any positive reason to suspect genetic abnormalities was used at its 10th subculture level and was designated as Non-Xp.

Growth and maintenance medium consisted of Eagle's minimal essential medium (MEM) with 10% foetal bovine serum, 100 units of penicillin and 50 µg kanamycin ml⁻¹.

One day before MNNG treatment, cells were trypsinised at their confluent monolayer stage in Falcon 250-ml plastic flasks and 20% of them were discarded, to obtain cells growing actively at the time of MNNG treatment. After growth for 24 h in an atmosphere of 5% CO₂ in air at 37 °C, cells were again trypsinised and resuspended into the original volume of medium containing MNNG at 1 × 10⁻⁵ M. MNNG was dissolved in a minimal amount of dimethyl sulphoxide and then growth medium was added to give the concentration which was equivalent to the 10% killing dose as judged by the nigrosin vital staining against Non-Xp cells under the conditions employed for MNNG treatment. After 60 min treatment with MNNG in a CO₂ incubator at 37 °C, cells were centrifuged at 750 r.p.m. for 10 min, washed with

fresh medium and then inoculated at 1 × 10⁶ cells per 15 ml medium per flask. The treated cultures were then grown in a CO₂ incubator with medium change twice a week. After 26 d incubation, cells were trypsinised to respread in the same flask, and further grown with a similar schedule of medium change. On the 32nd and 39th days, one flask of the cultures was subcultured into two flasks.

On the 43rd day the MNNG-treated CRL1200 culture produced several focus-like spots which still consisted of spindle-shaped cells, although their arrangement was altered and criss-crossed. The MNNG-treated Non-Xp cells showed a similar morphological alteration up to this stage but reverted to the normal arrangement soon after. The arrangement of some of these focus-like spots in the MNNG-treated CRL1200 culture only changed progressively and randomly oriented multilayers began to form around the centres of the spots on about the 63rd day. But constituent cells still had a spindle-shaped fibroblast appearance. On the 77th day multilayer formation at the foci became more and more marked until under a microscope cells on the top of each focus could no longer be observed simultaneously with the cells around the focus directly attached to the flask surface. Cells on the top of foci lost the fibroblastic appearance and were converted into small epitheloid cells with only one to two nucleoli, round or polygonal cytoplasm and an ovoid or round nucleus. The peripheral area around these foci was also altered to consist of a criss-crossed cell arrangement where giant cells supposedly formed by nuclear fusion were often observed, and made a marked contrast to the unaffected outer area which consisted of fibroblasts with monolayer orientation.

The multilayered foci were formed only in the MNNG-treated CRL1200 culture but never in the untreated CRL1200 nor in the MNNG-treated Non-Xp culture. These cultures were trypsinised on the 85th day and chromosome counts were made. Only the MNNG-treated CRL1200 culture with marked multilayered foci showed polyploidy and heteroploidy although the majority of cells in these cultures remained unaffected and grew normally as spindle-shaped cells. No marked change in chromosome counts was observed in the untreated CRL1200 culture.

On the 105th day after the MNNG treatment, cultures were trypsinised and distributed into several flasks at a cell concentration of 1 × 10⁴ per flask. Then the increase in cell number and the percentage of cells in the S phase (as demonstrated autoradiographically by daily incorporation of ³H-thymidine (0.2 µCi ml⁻¹) following 24-h pulse labelling) were examined. As shown in Fig. 1, the MNNG-treated CRL1200 culture showed a higher cell saturation density than did the MNNG-treated Non-Xp culture. DNA synthesis in the MNNG-treated CRL1200 culture was observed

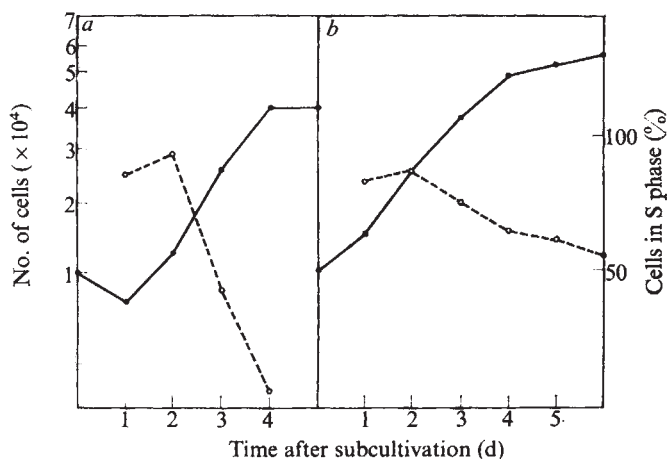


Fig. 1 Increase in cell number (solid line) and percentage of cells in the S phase (dotted line). a, Non-Xp; b, CRL1200 (Xp).

in 55% of the cells at saturation cell density but it was only 5% in the MNNG-treated Non-Xp culture. These observations suggested that the "transformed" CRL1200 culture, although still containing a large number of unaffected cells, consisted of cells showing "contact inhibition" less markedly than similarly treated Non-Xp cells. This was also confirmed in an autoradiogram which demonstrated that many of the cells constituting multilayered foci were in the S phase and that even the peripheral cells with monolayer orientation and fibroblastic appearance were actively synthesising DNA whereas unaffected cells incorporated almost no ^3H -thymidine label. Similar results to those described above for CRL1200 were obtained in another Xp strain, Xp-T.

These MNNG-treated transformed cells were examined three times for malignancy by transplantation into athymic nude mice which resulted in regression. Although tumorous growth was initially observed, histologically no sarcomatous pattern was shown. If the above described alteration in Xp cells after treatment with MNNG can really be considered as transformation, then it follows that only the Xp cells can be transformed by treatment with MNNG under the conditions employed but Non-Xp cannot. It had already been reported⁷ that cells from an Xp patient showed a higher frequency of transformation by SV40 than cells from the mother of the patient heterologous for the Xp gene. These observations suggest the interesting possibility of a common biochemical step between viral and chemical carcinogenesis and that this step itself is somehow affected in Xp patients.

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Distribution and valency of receptor for IgE on rodent mast cells and related tumour cells

MONOMERIC IgE binds tightly to the surface of mast cells and basophils without, by itself, causing any known perturbation. Interaction of the IgE with an appropriate antigen (or experimentally with anti-IgE) causes the cells to degranulate. Little is known about the initial molecular events in IgE-mediated triggering, but there is considerable evidence that crosslinking of the IgE molecules is a critical step (see refs 1 and 2 for discussion). The available data suggest that only limited bridging rather than an aggregation-induced extensive redistribution of the surface IgE^{3,4} is required; indeed if the bridging is extensive enough to induce such gross redistribution within the time that degranulation would ordinarily be expected, the latter is inhibited^{3,5}. Since bridging of the IgE, and therefore of the receptor to which it is bound, seems to be a critical signal, it is important to know if the cellular binding sites for IgE are integrated into larger functional units; the receptor molecules themselves

might be multivalent, or individual receptors might be connected to each other by some other component (Fig. 1). The experiments described here were directed towards investigating this point. We saturated cells with a mixture of two distinguishable types of rat IgE and determined whether by redistributing one of them with specific antibody, the other would comigrate.

Rat IgE was purified⁶ from the ascitic fluid of rats bearing the IgE-producing immunocytoma IR162 (ref. 7). Fluorescein-labelled IgE (F-IgE) was prepared by reacting 10 mg ml⁻¹ of IgE with 2 mg ml⁻¹ fluorescein isothiocyanate, adsorbed on celite⁸ at pH 9.6 for several days. After centrifugation the solution was passed over Sephadex G-25. The final preparation which was fully active (as determined by comparing it with native IgE for its capacity to inhibit the binding of ¹²⁵I-IgE) had an average of 12 mol fluorescein per mol protein. Rhodamine-conjugated IgE (R-IgE) was prepared in an analogous manner (by Dr J. Schlessinger) and was similarly fully active.

The antisera used were as follows: rabbit anti-rat IgE was prepared as described previously⁹; anti-fluorescein antibodies were prepared from horse anti-fluorescein serum¹⁰ (the gift of Dr D. W. Scott) by adsorption on a column of fluoresceinated bovine IgG-Sepharose 2B and, after washing, elution with 0.1 M acetic acid. Rabbit anti-fluorescein antibodies were prepared from rabbit anti-fluorescein serum¹¹ (the gift of Dr R. Cathou) in exactly the same way. Comparable results were obtained with both anti-fluorescein sera. The preparation of fluoresceinated sheep anti-rabbit IgG has been described previously³. The four types of cells studied were: normal rat mast cells, rat basophilic leukaemia cells, normal mouse mast cells and mouse mastocytoma cells (AB-CBF₁-MCT-1); their preparation has been described previously¹².

The principal experiments performed involved incubating the cells with a mixture (1:1-3:1) of F-IgE and R-IgE (total IgE concentration 10-15 $\mu\text{g ml}^{-1}$) with $\sim 1 \times 10^6$ cells ml⁻¹ for 60 min at 37 °C. The cells were washed once, taken up in one-tenth of the volume and reacted with $\sim 10 \mu\text{g}$ of anti-fluorescein (or anti-IgE) for 30-60 min at 37 °C and then examined with a Leitz Orthoplan microscope equipped with a Phloem incident light attachment, BG38 and KP490 exciting filters and a K530 suppression filter.

Cells pretreated with an excess of unconjugated IgE and then incubated as described always showed negligible green or red fluorescence. Cells reacted with the fluorescent IgE mixture and no anti-fluorescein or anti-IgE showed a diffuse distribution of both fluorescent chromophores (Table 1, experiment 1). The green fluorescence on cells reacted with the mixture of F-IgE and R-IgE and then with anti-fluorescein (Table 1, experiment 2) was distributed in multiple

Fig. 1 Three basic models of IgE receptor valency or integration which were explored by the experiments reported here. *a*, Receptors univalent and independent; *b*, receptors multivalent and independent; *c*, receptors univalent and connected. The experiments did not distinguish between *b* and *c* or a combination of *b* and *c*.

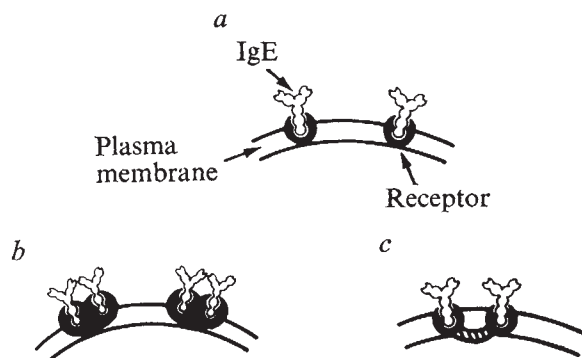


Table 1 Distribution of fluorescent IgE on rat and mouse cells

Type of experiment	Preincubation	Antiserum	Chromophore on IgE*	Topological distribution	
				Normal cells	Tumour cells
1	F-IgE + R-IgE	—	F R	diffuse diffuse	diffuse diffuse
2	F-IgE + R-IgE	anti-fluorescein	F R	small patches, vesicles diffuse	small patches, vesicles diffuse
3	F-IgE + R-IgE	anti-IgE	F R	comigration of F-IgE and R-IgE in small and large patches	comigration of F-IgE and R-IgE in caps and large patches
4	F-IgE	anti-fluorescein followed by R-IgE	F R	— —	small patches, vesicles† diffuse†
5	F-IgE	anti-IgE followed by R-IgE	F R	— —	caps and large patches, some vesicles† comigration with F-IgE plus diffuse†

*F, Fluorescein; R, rhodamine.

†Only mouse mastocytoma cells tested.

small irregular patches which appeared to be on the surface of the cells, as well as multiple smooth-bordered vesicle-like forms which appeared to be inside the cells. Some of the latter had a non-fluorescent centre, giving a "doughnut-like" distribution of fluorescence. In some cases the patches and vesicles were distributed asymmetrically on and in the cell but rarely were larger patches or caps observed. There was never any redistribution of the red fluorescence (R-IgE); cells reacted with anti-fluorescein or buffer were indistinguishable in this regard.

Cells reacted with NaN_3 (0.2 M) or at 4 °C during incubation with anti-fluorescein showed the F-IgE to be distributed in patches, but no vesicular forms were observed. When cells were first reacted with anti-fluorescein at 4 °C and the temperature was then raised to 37 °C, the vesicles appeared and became maximal within 15–20 min. As before, the red fluorescence never showed any redistribution.

Cells incubated with the F-IgE and R-IgE mixture and then with anti-IgE (Table 1, experiment 3) showed a different pattern; in all cases, the F-IgE and R-IgE comigrated. The basophilic leukaemia cells and mastocytoma cells showed extensive capping and large patch formation. The rat mast cells showed small or larger patches and only very rarely a fluorescent cap. The small mouse mast cells showed some cap formation but the larger cells showed only coarse aggregation. Vesicle formation was rarely seen with any of the cells. A similar picture was seen with cells reacted with IgE (unconjugated or conjugated), rabbit IgE and fluorescent sheep anti-rabbit IgE: the tumour cells showed large patches and caps whereas the mast cells showed principally patch formation; again little vesicle formation was observed.

These experiments demonstrated that receptors binding R-IgE did not comigrate with the receptors occupied by F-IgE when the cells were treated with anti-fluorescein, but did so when reacted with anti-IgE. We used mouse mastocytoma cells to test what would happen to empty receptors when receptors with IgE bound to them were redistributed. Cells were reacted with F-IgE such that when an aliquot was tested with ^{125}I -IgE only 40% of the sites were found to have been saturated. The partially saturated cells were reacted with antibody for 1 h at 37 °C, washed once and then reacted with excess R-IgE. Cells reacted with anti-fluorescein (Table 1, experiment 4) showed marked vesicle formation and some small patches for the green fluorescence but the red fluorescence was diffusely distributed. The green fluorescence on cells reacted with anti-IgE (Table 1, experiment 5) showed marked capping, and a small amount of vesicle formation (this was the only experiment in which vesicle formation was observed with anti-IgE). The R-IgE partially comigrated with the F-IgE

but was also diffusely distributed in areas between the green patches. We interpret this as the result of binding of R-IgE to non-patched empty receptors as well as to empty combining sites on anti-IgE in the patches formed by anti-IgE complexed to F-IgE. Control cells untreated with antibody showed a diffuse distribution of both F-IgE and R-IgE.

The results of our experiments are consistent with the observations on the surface distribution of IgE as assessed by transmission electron microscopy using ferritin-labelled antibodies^{3,5,13}. Those studies showed that in conditions in which the antibody itself would not be expected to perturb the system, the IgE was diffusely distributed. Although it was difficult to rule out oligovalency of the receptors, there was no evidence for even bivalency, and multivalency was clearly not observed. Those experiments could not of course elucidate whether there was any connection between receptors (Fig. 1c).

The present results seem, however, to be in conflict with those of Ishizaka and Ishizaka¹⁴, who carried out the following experiment: human basophils partially saturated with human IgE were reacted with anti-IgE in conditions which led to gross surface redistribution of the IgE. The cells were acidified in conditions where a substantial proportion of the cell-bound IgE-anti-IgE complexes seemed to be dissociated. The neutralised cells were then reincubated with radioiodinated IgE and the surface distribution of the radiolabelled IgE examined by autoradiography. Another portion of the cells was first saturated by added unlabelled IgE before the addition of the anti-IgE. Significantly, the two populations of cells showed a similar distribution of grains, over 50% of the cells showing either a capped or grossly asymmetric distribution of the label. These results, which suggested comigration of 'empty' receptors with receptors to which IgE was bound, were interpreted as "... that a group of receptor sites are associated with each other at the cell membrane or that receptor molecules on [the] basophil membrane are multivalent with respect to the combining sites for IgE"¹⁴. That is, models 1b and/or 1c were favoured: our data are only consistent with 1a (Fig. 1) and we are uncertain about the reason for this apparent discrepancy. Among the possibilities are (1) that the difference results from the use of cells from different species (human as opposed to rodent); (2) the use of different cells (basophils as opposed to mast cells); (3) that in spite of having several controls, the experimental results result from some artefact introduced by the nature of the protocol. The relative simplicity of our own experiments make us doubt that this has occurred in our studies, and so we conclude that, at least with regard to rodent mast cells

and related tumour cells, the receptors for IgE conform to the model depicted in Fig. 1a. We are not suggesting that the receptors are not interacting with other (sub)-membranous structures; indeed some results of studies on the diffusion of bound IgE in the plane of the membrane suggest that this may be true¹⁵. In the study by Schlessinger *et al.*¹⁵ it was similarly shown that the receptors for IgE are independently mobile and are monovalent.

Using non-ionic detergent it is possible to solubilise the cell-surface receptor for IgE in an active form¹⁶. Recent results indicate that such receptors are univalent with regard to their binding of IgE¹⁷. Together with the results reported here, these findings indicate that the receptor *in situ* is similar to the solubilised receptor, at least with regard to this important property.

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Lateral motion and valence of Fc receptors on rat peritoneal mast cells

MAST cells and basophils bind monomeric IgE antibodies reversibly but tightly by way of specific Fc receptors on their surface. Limited bridging of bound IgE molecules by multivalent antigen or anti-IgE antibody releases stored granules which liberate histamine and other mediators of the allergic response¹. The interaction of receptor-bound IgE with antigen (or with anti-IgE antibody) and the consequent cellular response (degranulation) provides an excellent opportunity for exploring the role of the lateral mobility of defined receptors in the transmission of 'signals' across the plasma membrane. Degranulation requires a limited bridging of complexes; excessive aggregation inhibits this response^{2–4}. We therefore measured the lateral mobility of the unperturbed IgE–receptor complexes on rat peritoneal mast cells and the effects on mobility of conditions that induce or inhibit degranulation. We redetermined⁵ the valence of the IgE receptor and the extent to which the IgE–receptor complexes react with each

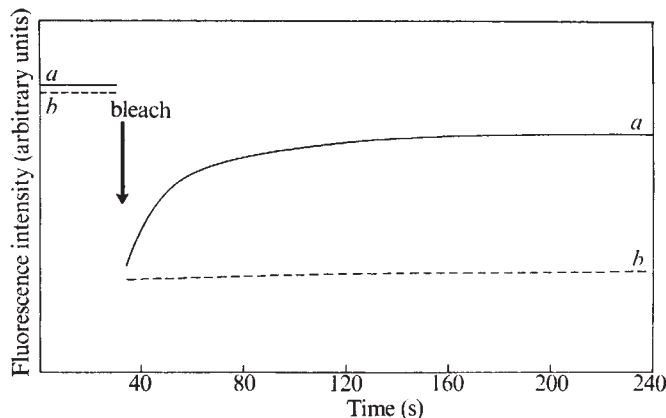


Fig. 1 Photobleaching recovery curves of mast cells labelled with *a*, R-IgE, $D = 1.8 \times 10^{-10} \text{ cm}^2 \text{ s}^{-1}$, f.r. = 75% (—); *b*, R-IgE and anti-IgE antibodies $D < 6 \times 10^{-12} \text{ cm}^2 \text{ s}^{-1}$, f.r. < 10% (---). In all experiments the small illuminated spot was exposed for 0.5 s to laser power of $\sim 1 \text{ mW}$. $D = (w^2/4\tau_{1/2}) \gamma$ where w is the e^{-2} radius of the focused Gaussian laser beam (1 μm); γ accounts for the amount of bleaching and the beam profile. In these experiments $\gamma \approx 1.3$ –1.4 (ref. 8). Similar D and fluorescence recovery values were obtained for laser beam focused on the top or the bottom of the settled mast cells. Fixation with 3% glutaraldehyde for 1 h at 4 °C inhibited receptor motion.

other. Fluorescence photobleaching recovery (FPR) was used to measure the macroscopic lateral motion of fluorescent cell-surface components^{6–9}. We and others have used this or similar methods, to measure the mobility on cell surfaces of concanavalin A (con A) binding sites (which include various surface components) and nonspecifically labelled membrane proteins^{6,7,10–12}. The present work and a concurrent study of acetylcholine receptors on developing myotubes (Axelrod *et al.*, unpublished) are the first quantitative measurements of the lateral motion of specific cell-surface receptors.

The preparation of rhodamine- and fluorescein-labelled IgE (R-IgE and F-IgE) and of the antisera has been described elsewhere¹³, as has the procedure for obtaining the rat peritoneal mast cells¹⁴. The mast cells (at $10^6 \text{ cells ml}^{-1}$) were incubated with R-IgE or F-IgE or a 1:1 mixture of both, at a total IgE concentration of 20–30 $\mu\text{g ml}^{-1}$ for 45 min at 30 °C, then washed with Hanks' balanced salt solution (BSS), resuspended in 0.1–0.5 ml of BSS, and allowed to settle on glass microscope slides. In some experiments, 0.1 ml of mast cells preincubated with fluorescent IgE were treated with 10 μg of anti-fluorescein or anti-IgE antibody (sometimes in the presence of 0.01 M NaN_3 to prevent endocytosis) for 30 min at 37 °C, washed, and applied to a microscope slide. In FPR measurements a brief, intense, focused laser light pulse irreversibly bleaches the fluorescence in a small region ($\sim 3 \mu\text{m}^2$) of the membrane. The time course of recovery of fluorescence in the bleached region due to replenishment by fresh fluorophore from adjacent parts of the cell was recorded and the shape of the recovery curves showed that recovery is consistent with fluorophore diffusion on the cell surface⁸. Receptor

Fig. 2 Mast cells labelled with *a*, R-IgE; *b*, R-IgE and anti-IgE antibodies.



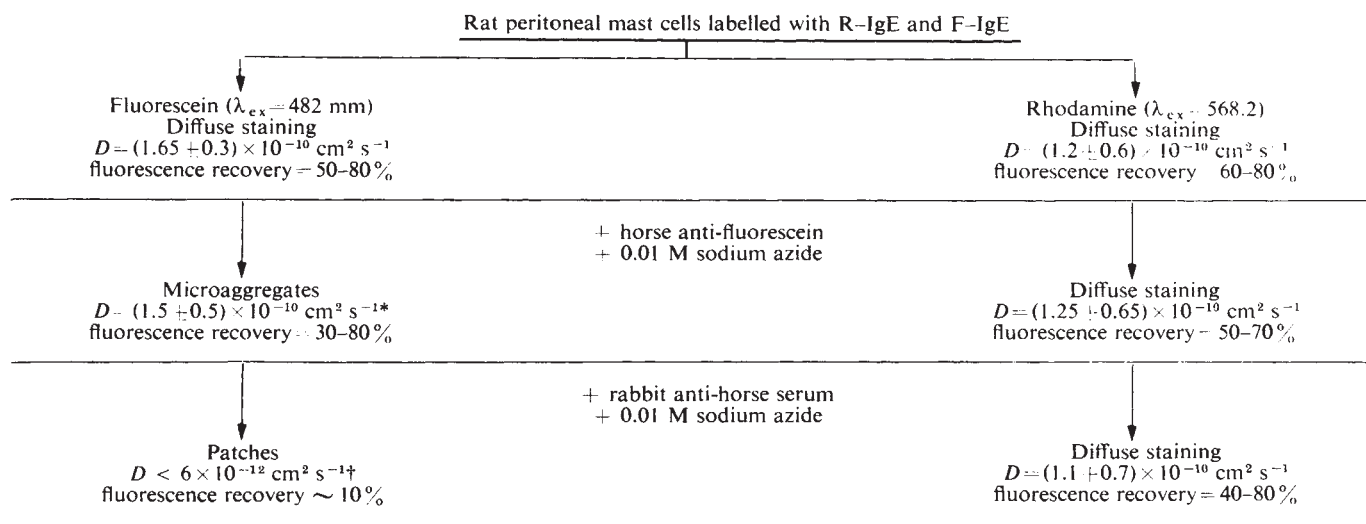


Fig. 3 Protocol for demonstration of IgE receptor valence. Mast cells were labelled with equal amounts of F-IgE and R-IgE. The values of D and f.r. and the fluorescence distribution were determined on individual cells independently for F-IgE and R-IgE by separate excitation of the two fluorophores using different excitation wavelengths. The left-hand column shows the protocol and results from detection of fluorescein; the right-hand column, from detection of rhodamine fluorescence on the same cell.

*Degranulation (G. Mendoza, unpublished).

†Inhibition of degranulation^{2,3}.

mobilities are, therefore, expressed as diffusion coefficients (D , $\text{cm}^2 \text{ s}^{-1}$) calculated from the recovery data. Incomplete fluorescence recovery is interpreted as indicating that a fraction of the fluorophores is immobile on the experimental time scale⁶⁻⁹. The fractional recovery (f.r.) therefore represents the fraction of mobile fluorophore⁶⁻⁹.

The mobility of dispersed IgE-receptor complexes is illustrated in Fig. 1, which shows a typical fluorescence recovery curve from a mast cell labelled with R-IgE. In this experiment, $D = 1.8 \times 10^{-10} \text{ cm}^2 \text{ s}^{-1}$ and the fraction of fluorescence recovery f.r. = 75%. The mean value and standard deviation for 17 cells was $D = (2.1 \pm 0.5) \times 10^{-10} \text{ cm}^2 \text{ s}^{-1}$ with f.r. in the range 50–80%. The division of the IgE-receptor complexes into mobile and immobile classes is interesting and although we cannot yet explain this heterogeneity, we have investigated factors which could influence it. We measured the diffusion coefficient of a fluorescent lipid probe (3, 3-dioctadecylindocarbocyanine iodide⁷) in the plasma membrane of mast cells and found $D \approx 8 \times 10^{-9} \text{ cm}^2 \text{ s}^{-1}$, 40-fold greater than that of the IgE-receptor complexes. The phospholipid matrix is thus quite fluid, as has also been observed in rat myoblasts^{6,7}. The presence of immobile Fc receptors suggests that more than the fluidity of the phospholipid bilayer controls the receptor mobility. Sodium azide (10^{-2} M , 30 min) had no effect on fluorescence recovery, suggesting that the expenditure of metabolic energy was not involved. Colchicine (10^{-5} M , 45 min), which inhibits microtubule assembly, seemed to reduce f.r. to 20–50% but had no effect on D . In contrast, cytochalasin B ($25 \mu\text{g ml}^{-1}$, 45 min), which inhibits microfilament assembly, dramatically reduced the effective diffusion coefficient of Fc receptor to $D = (3.4 \pm 0.8) \times 10^{-11} \text{ cm}^2 \text{ s}^{-1}$, and reduced the f.r. to 20–50%. These three substances do not affect the diffusion of a lipid probe in either myoblast plasma membranes or planar lipid bilayers, demonstrating that the viscosity of the lipid matrix was not altered by the drug treatments⁷. Therefore we conclude that cytoskeletal interactions, especially with microfilaments, can influence the receptor mobility^{6,7}.

Treatment of mast cells prelabelled with F-IgE with a 10- to 50-fold excess (over receptor) of anti-fluorescein antibodies causes degranulation (G. Mendoza, unpublished). FPR measurements of the diffusion of F-IgE in these conditions yielded $D = (1.5 \pm 0.5) \times 10^{-10} \text{ cm}^2 \text{ s}^{-1}$ with f.r. from 40% to

80%. Fluorescent microaggregates, each $< 1 \mu\text{m}$ incorporating a substantial part of the total fluorescence, were visible on the cell surface. Similar results were obtained two hours after labelling. Aggregation of IgE to a level adequate for degranulation did not therefore, cause a detectable change in mobility. We cannot, however, exclude effective immobilisation of a small fraction of F-IgE.

In contrast, incubation of cells with anti-IgE antibodies (rather than anti-fluorescein) following labelling with F-IgE or R-IgE caused extensive redistribution of the complexes and large patch ($> 1 \mu\text{m}$) formation (see Fig. 2). FPR measurements carried out soon (15 min) after labelling indicated that the crosslinked receptors were essentially immobile; $D < 6 \times 10^{-12} \text{ cm}^2 \text{ s}^{-1}$, f.r. $< 10\%$. Diffusion of receptors is thus unaffected by the limited crosslinking by anti-fluorescein that is adequate for degranulation. Diffusion is, however, strongly reduced in conditions in which degranulation is inhibited by excessive aggregation with anti-IgE^{2,3}.

In the accompanying paper, Mendoza and Metzger¹³ show that anti-fluorescein antibodies induced redistribution only of F-IgE cells prelabelled with both R-IgE and F-IgE and so they concluded that the Fc receptor is monovalent for IgE. We have devised another sensitive experiment (Fig. 3) to test this conclusion, in which mast cells were labelled with equal amounts of F-IgE and R-IgE. The mobility of the fluorophores could be measured separately using krypton laser lines at 482 nm and 568.2 nm respectively. As expected, horse anti-fluorescein antibodies affected the mobility of neither F-IgE nor R-IgE. Addition of rabbit anti-horse antibodies, which bind only to the anti-fluorescein antibodies, caused patching and abolished the mobility of the F-IgE without diminishing the mobility of the R-IgE (see Fig. 3). This reinforces the conclusion of Mendoza and Metzger¹³ that the Fc receptor is monovalent and rules out the possibility that individual receptor molecules interact or are bound together to a significant degree⁵.

The major conclusions of the present study are: (1) Most IgE-Fc receptor complexes are mobile with $D = (2.1 \pm 0.5) \times 10^{-10} \text{ cm}^2 \text{ s}^{-1}$, but 20–50% are immobile on the experimental time scale. (2) Limited crosslinking by anti-fluorescein that is adequate for degranulation did not affect the apparent mobility of the receptors. Extensive aggregation inhibitory of degranulation, however, abolished the mobility. (3) The Fc receptor is monovalent for IgE. (4) Cytochalasin B reduces the

lateral mobility of the complexes; colchicine has little detectable effect.

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Induction of an IgM anti-(bovine)-IgG response in mice by bacterial lipopolysaccharide

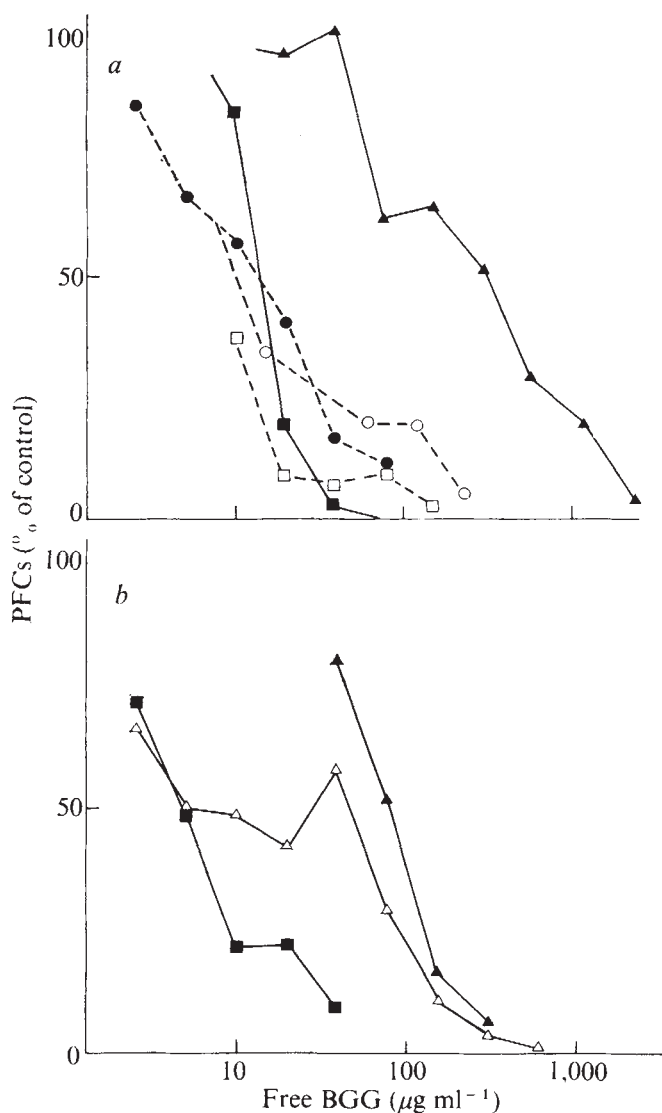
BACTERIAL endotoxin stimulates the appearance in mice of a large number of plaque forming cells (PFC) secreting an IgM globulin with specificity for bovine IgG (bovine gamma globulin; BGG). Reversed plaquing (antibody on the target erythrocyte) has enabled us to enumerate the total number of IgM secreting cells and show that the anti-BGG PFC comprise at least 10% of the total. Plaque inhibition with free antigen shows that anti-BGG PFC stimulated by LPS, as distinct from those specifically stimulated, secrete a product with low avidity. The evidence reported both here and in a previous study¹, leads us to suggest that substances such as LPS stimulate the production of an M-anti-G response. The rheumatoid-factor-like molecule which is produced may possibly serve to amplify complement fixation by high avidity/specificity IgG antibodies.

The injection into mice of certain substances apparently unrelated to sheep red blood cells (SRBC), can increase the level of 'background' antibody to SRBC, or the number of plaque forming cells producing antibody to SRBC²⁻⁴. Of such substances investigated, *Bordetella pertussis* (pertussis) organisms and water-in-oil emulsions containing heat killed mycobacteria (Freund's) can be readily classified as immunological adjuvants, and rabbit anti-(mouse)-lymphocyte globulin (ALG) as a mitogen: lipopolysaccharide endotoxins from Gram-negative bacteria (LPS) combine the properties of both⁴⁻⁶. LPS stimulates not only an increase in cell growth and division *in vitro* but also the development of cells secreting antibody to SRBC and to the haptens NIP and NNP⁷. Since the mitogenicity of LPS is

confined to B-lymphocytes and since it also stimulates the production of antibodies with a wide range of specificities, including those specific for the antigenic moiety of LPS itself⁸⁻¹⁰, Möller and his colleagues coined the term PCBA—polyclonal B-cell activator—to describe substances with the properties just ascribed to LPS¹¹.

In the course of experiments designed to investigate the induction of tolerance to BGG in CBA/Ca mice, some animals received either an intraperitoneal injection of alum adsorbed BGG plus pertussis or an intravenous injection of

Fig. 1 Inhibition of anti-BGG plaques by free BGG added to the medium of the plaque assay. Each assay consists of 0.2 ml 1% Agarose-Eagle's basal medium plus target erythrocytes, PFC, and developing serum, spread into an area of about 25 × 50 mm on a standard microscope slide. *a*, Solid lines refer to plaques developed by a μ -specific developing serum on slides carrying spleen cells from CBA/Ca mice injected intraperitoneally 4 d before with either 20 μ g LPS ($\blacktriangle$; 100% = 31×10^4 PFC/spleen) or intravenously with 1 mg HBGG ($\blacksquare$; 100% = 2.3×10^4 PFC/spleen). The dashed lines refer to three separate experiments in which plaques were developed with a γ_1 -specific developing serum¹⁶ using spleen cells taken 4 d after injection of HBGG (mean 100% = 1×10^4 PFCs per spleen). The solid symbols refer to data obtained on the same day. Each pool of spleen cells was prepared from five 8-10-month-old male mice. *b*, As for (*a*) except that all the data refer to anti- μ developed plaques. Mice were injected with either LPS ($\blacktriangle$; 100% = 7.5×10^4 PFC per spleen), HBGG ($\blacksquare$; 100% = 4.7×10^4 PFCs per spleen) or HBGG plus LPS ($\triangle$; 100% = 19.3×10^4 PFC per spleen).



heat aggregated BGG (HBGG). The immune response of these mice was measured using a haemolytic-plaque assay, using as target cells, SRBC and SRBC coated with BGG by the chromic chloride method^{12,13}. When a rabbit anti-(mouse)- μ serum was being used in an attempt to inhibit direct plaques, it was found that this serum would enhance by about fourfold the number of plaques against BGG coated SRBC: the concentration of anti- μ serum used was one which had already been shown to be highly inhibitory to direct plaque formation by anti-SRBC PFC from the spleens of actively immunised mice. The developed plaques, which are very small, are an example of indirect IgM PFCs^{14,15}.

Subsequently, it has been found that such indirect IgM anti-BGG responses can be stimulated in CBA/Ca mice by the injection of pertussis alone, and even more so by LPS (*Salmonella typhosa* 0901 B). The maximum response is found 4 d after an intraperitoneal injection of 20 μ g of LPS.

The (indirect) anti-BGG PFC stimulated by LPS are judged to be IgM-producers on the following criteria. (1) Their numbers are enhanced by a rabbit anti-(mouse)- μ serum, raised, absorbed and tested for specificity as described previously¹⁶. Another such serum, kindly provided by Dr R. M. E. Parkhouse, gave an identical enhancement, which was abolished by passage through a μ -bearing Sepharose 4B column. (2) A goat anti-(mouse)- μ serum has been prepared which inhibits totally both direct anti-SRBC and LPS stimulated anti-BGG plaques. The difference in behaviour between the rabbit and goat anti- μ sera may be explicable on the basis of their differing abilities to fix the guinea pig complement used in the assay¹⁷. (3) The indirect anti-BGG plaques are ablated by reduction and alkylation procedures designed to destroy IgM but not IgG¹⁴.

The anti-BGG response, nonspecifically induced by LPS, is restricted in duration and in Ig class, although the number of PFC present in the spleen at the peak of the response, 4 d after the intraperitoneal injection of LPS, is quite high ($>10^5$ PFC per spleen). In contrast, the anti-BGG response, specifically induced by intravenous injection of HBGG, produces far fewer PFC per spleen ($<10^3$), and in addition induced both an early IgG (γG_1) peak 5 d after the injection, and a late IgG (γG_1 , γG_{2b} , γG_{2h}) peak 10 d after the injection of HBGG. The avidity for BGG of the antibody produced by PFC in the two types of response, has been compared (Fig. 1). A relative avidity can be deduced from the amount of free BGG required to reduce plaque numbers by an arbitrary proportion¹⁸, say to 32% of the

numbers detected in its absence. All PFC, irrespective of class, which were specifically induced by HBGG are inhibited by far lower concentrations of BGG than are the indirect PFC induced nonspecifically by LPS: it is concluded that the antibody induced by LPS has a very low affinity (avidity) for BGG. It is interesting to note that immunisation by a mixture of LPS and HBGG leads to an exceedingly heterogeneous population of PFC as judged by the shallow slope of the inhibition curve (Fig. 1b). This suggests that although they may overlap extensively, LPS and HBGG tend to stimulate different bands in the spectrum of precursor cells capable of becoming anti-BGG PFC. The nature of the inhibition curves and the numbers involved provide evidence that LPS stimulates a very much larger number of precursor cells than does HBGG. The possibility that LPS and HBGG stimulate some of the same precursors cannot be excluded on the evidence.

It is debatable whether LPS triggers B lymphocytes through being bound to Ig receptors for antigen. With the proviso that the polysaccharide (antigenic) moiety probably behaves like any other antigen, the lipid A (adjuvant) moiety may bind either to Ig receptors for antigen at positions other than the antibody-like site or, alternatively, to other (non-Ig) sites on the cell membrane. The triggering reflex may be the result of a cumulative effect exceeding some unspecified threshold, which results from the binding and perhaps crosslinking of a variety of membrane components: antigen, LPS and certain factors of lymphocyte or macrophage origin may well help to exceed this threshold. An hypothesis which suggests that antigen and adjuvant give qualitatively different triggering stimuli to antigen sensitive cells (ASC), thereby deciding between tolerance and immunity, has been discussed elsewhere¹⁹.

In passing, it may be worth noting the following points additional to the apparent differences in relative avidity of the IgM responses to BGG. (1) HBGG but not LPS stimulates an IgG response. (2) Secondary IgG (IgM?) responses stimulated by BGG after priming with HBGG can be demonstrated, but it has not been possible to demonstrate that LPS stimulates a secondary IgM response in mice primed either by HBGG or LPS. (3) In "tolerance" induction large doses of BGG (>1 mg) are required to reduce the level of IgM response but much smaller doses (<10 μ g) to reduce the level of IgG response¹³. (4) The magnitude of the peak anti-BGG response stimulated by LPS increases with increasing age of the mice up to about 8 months and thereafter remains more or less constant. There are no

Table 1 The proportion of cells secreting IgM with antibody-like properties 4 d after an injection of LPS

Experiment number	Arithmetic mean number of cells or (IgM) PFC per spleen ($\times 10^4$)					
	Cells*	IgM secretors (indirect)	Anti-BGG (indirect)	Anti-sheep IgG (indirect)	Anti-TNP† (direct)	Anti-SRBC (direct)
Normal mice (not injected)						
1	11,600	13	0	0	1.7	0
LPS injected mice						
1	16,800	820	75	11	47	1.8
2	17,500	314	62	—‡	13	0.1
3	22,600	522	32	—	—	2.9
4	14,100	224	35	1.6	33	0.4
5	13,400	1054	34	—	—	1.1
6	10,700	—	24	—	—	—
7	10,800	874	—	—	—	—
8	13,800	178	13	—	—	0.4
Mean	14,963	569	39.3	—	31	1.1
($\pm$ s.e.)	(1,389)	(132)	(8.2)	—	(9.9)	(0.5)

*Cell counts were made with a model B Coulter counter windowed to exclude erythrocytes or smaller cells and cells the size of large blast cells or larger. As judged by Trypan blue exclusion cell viabilities are $>77\%$.

†SRBC coated with TNP at high epitope density by the method of Rittenberg and Pratt²¹.

‡A dash means that this target cell was not used in this experiment.

On the basis of these data, $4.0 \pm 0.43\%$ of spleen lymphocytes are secreting detectable amounts of IgM: of the IgM secretors $10.2 \pm 1.0\%$ are secreting anti-BGG antibodies, $8.2 \pm 1.9\%$ are secreting antibodies binding to TNP and only $0.22 \pm 0.07\%$ are secreting anti-SRBC antibody.

measurable changes in avidity to BGG of the IgM response induced by LPS over the age range of 2–20 months.

An estimate has been made of the total number of IgM secreting cells in the spleens of mice, both before and after the injection of LPS. The estimate was made using the technique of "reversed-plaquing"²⁰ in which the target erythrocytes used in the plaque assay were coated with IgG from a goat anti-(mouse)- μ serum. An essential step was to develop the plaques by use of a rabbit anti-(mouse)- μ serum at the concentration used to enhance the level of anti-BGG plaques. Table 1 summarises the results of several experiments in which estimates have been made of the number of IgM secreting cells and the proportion of these cells which were secreting antibody reactive with BGG, sheep IgG, SRBCs and the hapten 2,4,6-trinitrophenyl (TNP). It is striking that, on the basis of these measurements, the anti-BGG response comprises about 10% and the anti-TNP about 8% of the total number of IgM secreting cells.

We think it is possible that the IgM response to BGG induced by an injection of LPS represents an unconventional immune response in which rheumatoid-like factors^{22,23} are secreted in response to stimulation by toxic products released by bacteria. In favour of this hypothesis are the facts that the response is IgM, low avidity and directed against an IgG molecule. The implication would be that to the CBA mouse, bovine IgG and bound or denatured murine IgG have similar antigenic determinants. So far however our attempts to demonstrate this directly using the plaque assay have been inconclusive. Nevertheless it has been possible to demonstrate that LPS stimulates the production of a humoral IgM (19S) which binds with relatively low avidity to mouse (γ G_{2a}) antibody bound to antigen (SRBC)¹. If our reasoning is correct, the high proportion of IgM secreting cells whose products have anti-IgG activity, could be regarded as a fast amplification mechanism which might be an adjunct to the complement cascade. The selective pressures influencing the evolution of this mechanism would therefore be quite different from those moulding high avidity responses to foreign determinants with a wide range of specificities.

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Immunological response restricts number of cells in sensory ganglia infected with herpes simplex virus

INFECTION of mice with herpes simplex virus (HSV) by various epithelial routes has been shown to result in a latent infection of the corresponding sensory or autonomic ganglia^{1–3}. During the acute phase of the infection (less than 14 d), virus could be recovered from ganglia both by homogenisation and explantation, while during the latent phase of the infection (21 d to more than 1 yr), virus could be recovered only by explantation and not by homogenisation. Although these methods made it possible to tell whether ganglia were infected, they gave no information about the percentage of ganglionic cells containing virus. We have therefore investigated the number of cells in sensory ganglia infected with HSV, and evaluated the influence of immunity on the severity of the infection.

HSV, type 1 (strain CHR-3), containing 2×10^8 plaque-forming units (PFU) per ml was prepared and assayed in primary rabbit kidney (PRK) cells⁴. Swiss athymic nude mice (Nu/Nu), normal littermates (Nu/+ or +/+) and BALB/c mice were infected with HSV by administering undiluted stock virus to the abraded right rear footpad⁵. BALB/c mice were immunised with HSV by administering increasing doses of infectious virus (10^3 – 10^6 PFU) intraperitoneally at 10–14-d intervals, over 2 months. Previous experiments showed that the dorsal root ganglia (DRG) of mice did not become infected as a result of intraperitoneal immunisation⁵. Hyperimmune serum to HSV was prepared in rabbits by repeated intradermal injection (0.5 ml) of partially purified virus⁶. Neutralisation titres are expressed as the reciprocal of the highest dilution of serum producing a 50% reduction in viral plaques.

To determine the number of ganglionic cells infected with HSV, DRG were removed, washed in Dulbecco's phosphate-buffered saline (PBS) and incubated at 36 °C for 15 min in 2.0% collagenase (approximately 10 ganglia per ml). Tissue was aspirated gently four or five times with a Pasteur pipette, washed three times in trypsin-EDTA (0.25% trypsin in 4×10^{-4} M EDTA-saline), and then incubated at 36 °C for 15 min in the trypsin-EDTA solution. Foetal bovine serum was added to a final concentration of 10%. To eliminate extracellular virus, the ganglionic cells were

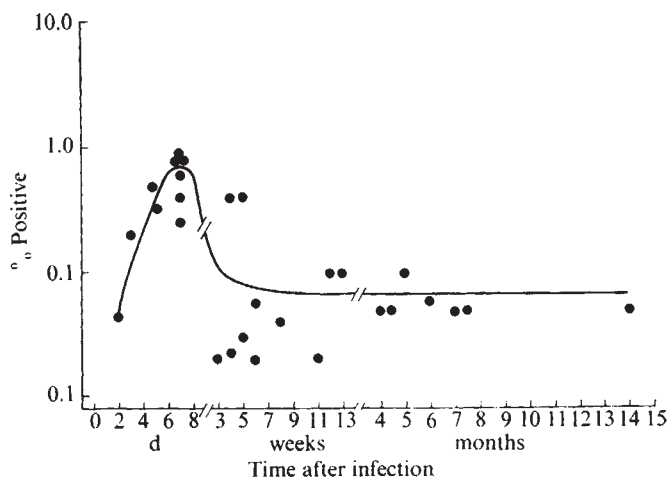


Fig. 1 Percentage of ganglionic cells infected with HSV at different times after infection. BALB/c mice were inoculated with HSV by the footpad route and DRG were removed and dissociated into single cells. Serial twofold dilutions of the cell suspension were placed on PRK cell monolayers and the percentage of infected ganglionic cells was determined.

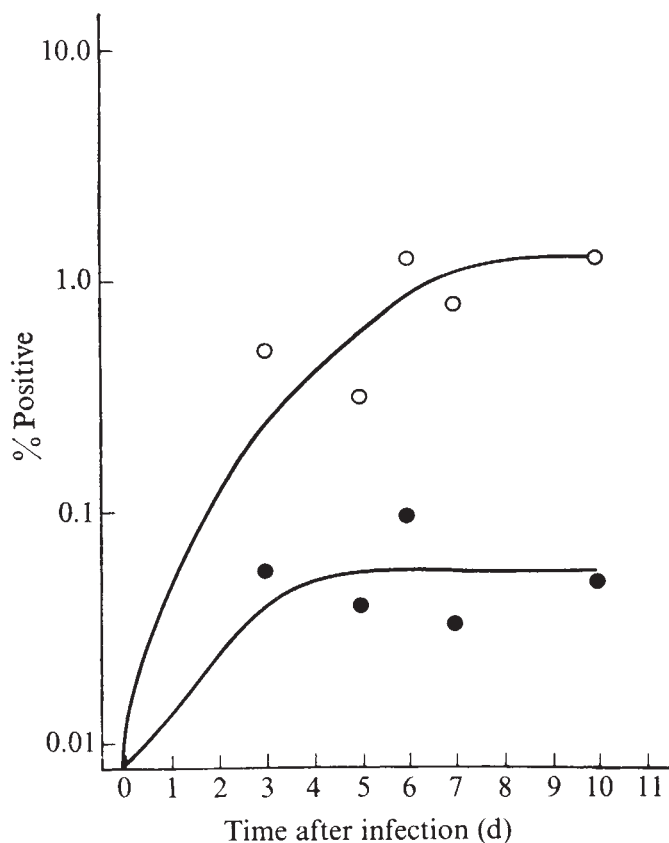


Fig. 2 Effect of immunisation on number of cells in DRG infected with HSV. Immunised (●) and unimmunised (○) BALB/c mice were inoculated by the footpad route with HSV and at different times thereafter DRG were removed and dissociated and the percentage of infected cells was determined.

resuspended and incubated for 30 min in rabbit anti-HSV serum (50% neutralisation titre, 400). The cell suspension, containing primarily single cells, was washed five times to remove excess neutralising antibody, resuspended in PBS, and the viable cells were counted by Trypan blue exclusion. Based on staining for Nissl bodies⁷, we estimated that the final cell suspension contained 5–10% neurones. Supernatant fluid from the fifth cell wash was assayed to ensure that infectious virus was not present in the final cell suspension. Serial twofold dilutions of the cell suspension were placed on triplicate or quadruplicate monolayers of PRK cells. Cultures were examined daily for 10 d for the appearance of HSV cytopathology. Percentage of ganglionic cells infected was calculated (according to the method of Reed and Muench) by determining the number of dispersed ganglionic cells required to produce lysis of the PRK monolayers in 50% of the cultures (that is, based on the assumption that only one infected ganglionic cell was needed to initiate lysis).

DRG were removed from animals at different times after footpad inoculation with HSV, and the number of infected cells in ganglia was determined. Figure 1 shows that between 6 and 8 d after inoculation, almost 1.0% of the cells in the ganglia were infected. During the next few weeks, the percentage of infected cells decreased to 0.1% or less, and remained at that level for almost 15 months.

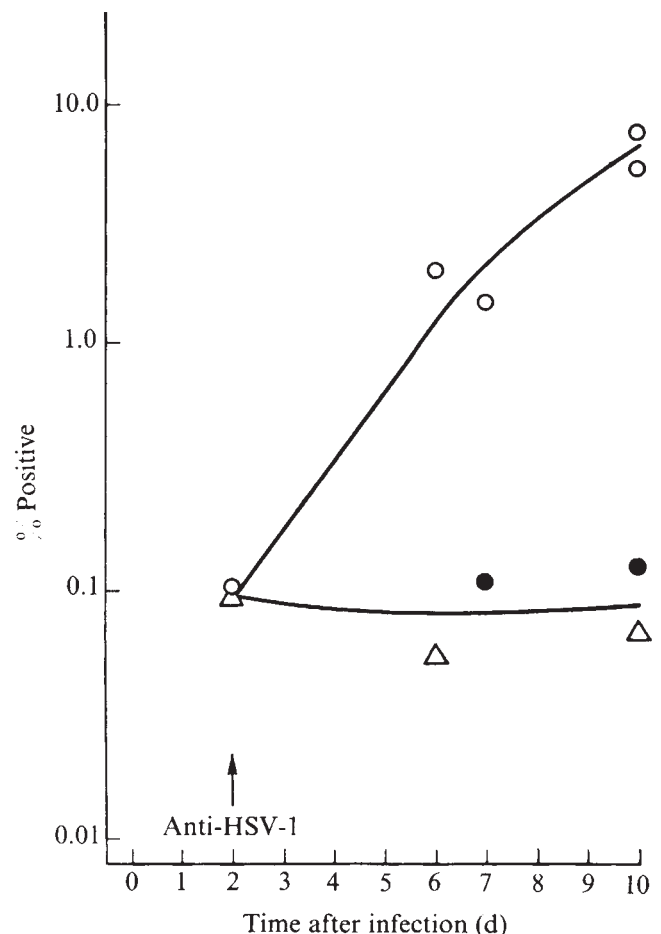
Earlier experiments showed that immunisation with HSV did not prevent mice from developing a latent infection when challenged by the footpad route³. To see whether immunisation affected the total number of ganglionic cells that became infected, immunised and unimmunised mice were challenged with HSV by the footpad route, and at different times thereafter the virus-infected cells in ganglia were counted. The data in Fig. 2 show that immunisation

restricted infection of ganglionic cells; approximately one-tenth the number of ganglionic cells was infected in immunised as in unimmunised animals. Moreover, the number of infected cells in ganglia of immunised animals during the acute phase of infection corresponded closely to the number of infected cells in ganglia of unimmunised animals during the chronic phase of the infection (Fig. 1).

Further evidence that the immune status of the host influences the number of cells infected in ganglia comes from studies with nude mice. Figure 3 shows that the number of infected cells in ganglia of nude mice (Nu/Nu) continued to rise during the first 10 d of the infection and reached close to 7.0%. In contrast, only 0.1% of ganglionic cells was infected in nude mice given antibody to HSV 2 d after HSV inoculation. Similarly, only 0.1% of the ganglionic cells was infected in phenotypically normal littermates of nude mice (Nu/+ or +/+) not given antibody to HSV. Thus, the nude mouse, which cannot mount an effective immune response to HSV⁸, has almost 70 times more HSV-infected cells in the sensory ganglia than the antibody-reconstituted nude mouse (Nu/Nu) or the phenotypically normal mouse (Nu/+ or +/+).

The studies described here show that by dissociating sensory ganglia, it is possible to determine the number of cells

Fig. 3 Percentage of ganglionic cells infected with HSV in immunologically deficient animals. Nude mice (Nu/Nu) and phenotypically normal littermates (Nu/+ or +/+) were inoculated by the footpad route with HSV. Two days later the nude animals were given intraperitoneally either 0.1 ml of normal mouse serum or 0.1 ml of mouse anti-HSV serum (50% neutralisation titre, 1,048). At different times thereafter DRG were removed and dissociated and the percentage of infected cells was determined. ○, Nude mice given normal mouse serum; ●, nude mice given mouse anti-HSV serum; △, phenotypically normal littermates of nude mice.



infected by HSV. Because of the potentially low plating efficiency of infected cells, as well as the possible loss of cells due to physical manipulation, the actual number of virus-infected cells in ganglia may be considerably greater than the 0.1–1.0% obtained in our experiments. Moreover, although our data are expressed as percentage of cells in ganglia infected with HSV, it was estimated that less than 10% of the cells plated were neurones.

Even though the assay used here may greatly underestimate the true number of cells infected, it does provide a useful method for comparing the relative number of cells infected in different experimental conditions. In this connection, we found that the number of infected ganglionic cells fell off sharply after the acute phase of the infection and remained relatively constant for many months thereafter. Whether this is due to two populations of cells in the ganglion susceptible to HSV—one capable of being lysed by the virus, and the other capable of maintaining a latent infection—or whether the immune response of the host destroys some of the virus-infected cells is not known. Our studies with immunised and nude mice, however, do show that the immunological status of the host is one of the factors that controls the number of cells in ganglia that becomes infected. Precisely how the immune response of the host restricts infection of ganglionic cells is not clear. It is known that HSV replicates in the skin and travels up the nerve to the cell body in the ganglion^{9–11}. Thus the number of nerve endings exposed to the virus may determine the number of cells ultimately infected. If the immune response of the host inhibits viral replication in the skin, the number of cells becoming infected would be reduced correspondingly. Alternatively, the virus may spread from one cell to another within the ganglion and the humoral and/or cellular immune response may act at this level¹². The demonstration in nude mice that passive administration of antibody is protective, suggests that humoral immunity plays a part in limiting the initial spread of the virus. Whether humoral immunity also is responsible for maintaining the latent state of the infection¹³ cannot be resolved from our experiments.

The demonstration that, in different experimental conditions, the number of infected ganglionic cells varies considerably, suggests that this might be an important factor in determining the subsequent clinical course of the disease. All other things being equal, the severity and frequency of recurrent HSV attacks might very well be related to the number of latently infected cells.

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A human chromosomal determinant for susceptibility to herpes simplex virus

THE susceptibility of mammalian cells to virus infection has been studied in many virus–host systems. In certain instances^{1–6}, the comparison of cells which do not permit productive infection with those that do has made it possible to identify the host factors which allow entry of the virus and support its subsequent growth. In the case of poliovirus the use of somatic cell hybrids between permissive and non-permissive cells has led to the assignment to human chromosome 19 of a gene coding for virus susceptibility⁷. Although cells from a wide variety of organisms, including many rodents and man, are susceptible to lytic infection by herpes simplex virus type 1 (HSV-1) resistance to HSV-1 infection has been reported in certain Chinese hamster cell lines^{8–10}, in dog kidney cells¹¹, and in a line of rat cells¹². In these cell lines HSV-1 is either non-infectious or exhibits virus titres several orders of magnitude lower than in Syrian hamster or rabbit cells. The cellular factors involved in this resistance to infection and in the genetic control of susceptibility to HSV-1 have not yet been identified.

We report here experiments in which Chinese hamster × Syrian hamster and Chinese hamster × human somatic cell hybrids were used as hosts for HSV-1 infection. The results indicate that susceptibility to HSV-1 infection behaves as a partially dominant genetic trait in these cells, and that its behaviour in Chinese hamster × human hybrids which are segregating human chromosomes is consistent with its being determined by the presence of human chromosome 3.

The results in Table 1 confirm the earlier finding⁹ that HSV-1 has low infectivity in Chinese hamster Don cells¹³ and show that genetically marked derivatives of Don are also less susceptible to HSV-1. As certain of the cell lines were unable to form complete monolayers, we used the 17 *syn* strain¹⁴ of HSV-1 in most of our experiments. This strain causes polykaryocytosis which results in the formation of easily recognised syncytial plaques. Where tested, essentially similar results were obtained with the *syn*⁺ parent strain. As can be seen, virus titres were at least 10⁴-fold lower on Don and its derivatives than on Syrian hamster BHK-C13 cells¹⁵. Reproducible virus titres on Don cells could only be obtained at multiplicities above 0.1 PFU per cell (as measured on BHK-C13 cells). The burst size in these conditions as measured by infectious centres and yield assays (approximately 150 PFU per infected Don cell) was comparable to that on BHK-C13 cells. The progeny virus

Table 1 Susceptibility of Syrian hamster and Chinese hamster cell lines to HSV-1 strains

Cell line	Virus titre (log ₁₀ PFU* ml ⁻¹)	
	HSV 17 <i>syn</i>	HSV 17 <i>syn</i> ⁺
BHK-C13	7.3	9.3
C13 TK ⁻ †	7.3	9.6
Don	2.3	5.2
Don TG 5.10‡	2.5	5.6
Don TK ⁻ (a23)§	2.5	ND

Monolayers of each cell line in 50-mm plastic Petri dishes were infected with HSV 17 *syn* (low titre stock passaged in Lesch-Nyhan GM29 cells) or HSV 17 *syn*⁺ (high titre stock passaged in BHK-C13 cells). After 40 min incubation at 37 °C in Glasgow-modified Eagle's medium supplemented with 10% foetal calf serum, the inoculum was neutralised by the addition of an equal volume of medium containing 10% human serum. The neutralised inoculum was removed after a further 20 min incubation at 37 °C and replaced by medium containing 10% human serum. Incubation was continued for a further 48 h at 37 °C. Dishes were stained with Leishman stain and virus plaques counted. ND, not determined.

*Plaque-forming units.

†Isolated by Littlefield and Basilico¹⁶.

‡Isolated in this laboratory from Don cells by selection in 5 µg per ml 6-thioguanine.

§Isolated by Dr A. Westerveld¹⁸, and supplied by him.

Table 2 Susceptibility of Syrian hamster $\times$ Chinese hamster somatic cell hybrids to HSV-1

Clone	Virus titre (log ₁₀ PFU ml ⁻¹)	Modal chromosome no.	Don contribution to hybrid cells
BHK-C13 TK ⁻	7.2	41 (36-45)*	—
Don TG 5.10	2.7	44 (42-46)†	—
A1	5.0	60 (45-65)	1s‡
A5	5.3	65 (65-72)	1s
A6	4.6	62 (55-71)	1s
B3	5.0	100 (88-104)	1s
B5	5.3	61 (55-65)	1s
C1	6.0	86 (75-95)	1s

BHK-C13 TK⁻ and Don TG 5.10 (HGPRT⁻) cells were fused in monolayer¹⁷ using ultraviolet-inactivated Sendai virus (Searle Diagnostic Ltd), and complementing hybrid colonies isolated in modified²⁰ HAT medium¹⁸. Independent colonies were picked after 14 d, grown in plastic flasks (Nunc) to a total population size of 5×10^7 cells, and aliquots taken for karyotype and isozyme analyses or for storage in liquid N₂. Virus susceptibility tests were performed using HSV 17 syn as described in Table 1, on thawed cells which had been grown for one further passage. Chromosome numbers and the complement of Don chromosomes were determined by examination of Giemsa-banded²⁰ metaphase spreads.

*Range of values (90% limits).

†Don TG 5.10 is tetraploid.

‡1s indicates the presence of not more than 44 Chinese hamster chromosomes in the majority of cells.

produced did not, however, show an increased titre on Don cells.

The molecular basis for the low susceptibility of Don cells to HSV-1 infection is at present being investigated. Results not shown here indicate that, although adsorption and penetration of virus occur normally, there is little or no virus eclipse and viral thymidine kinase is not induced. We cannot at present, however, exclude the possibility that some early viral proteins are synthesised in Don cells infected at low multiplicity.

In order to determine whether susceptibility to HSV-1 is a genetically dominant trait, somatic cell hybrids were made between non-permissive and permissive cells. Cells of a Don HGPRT⁻ variant (Don TG 5.10) and a BHK-C13 TK⁻ variant were fused and complementing hybrids between them isolated under conditions designed to provide a series of hybrid clones of independent origin. These clones were tested at low multiplicities for their susceptibility to HSV-1. Table 2 shows that the Don $\times$ BHK hybrids were susceptible to HSV-1 and gave virus titres 10^2 – 10^3 -fold less than the BHK parent. This result indicates that susceptibility

Table 3 Susceptibility of Chinese hamster $\times$ human somatic cell hybrids to HSV-1

Clone	Virus titre (log ₁₀ PFU ml ⁻¹)	Susceptibility
Parental lines		
GM29*	6.5	+
Don TK ⁻ (a23)	2.3	—
Hybrids		
GMA A1.5	2.8	—
GMA A2.5	4.2	+
GMA C1.3	2.5	—
PF 1B6	<2.0	—
PF 2A5	4.3	+
PF 2B1	3.3	+
PF 2B2	3.3	+
PF 2C2	<2.0	—
PF 4A1	<2.0	—
PF 4A2	4.0	+
PF 4A6	<2.0	—
PF 4B1	<2.0	—
PF 4B2	4.6	+

Lesch-Nyhan GM29 (HGPRT⁻) and Don TK⁻ (a23) cells were fused in monolayer (PF series) or suspension (GMA series) using ultraviolet-inactivated Sendai virus¹⁷. Complementing hybrids were isolated and analysed as in Table 2.

*Purchased from the Mammalian Genetic Mutant Cell Repository, Camden, New Jersey.

to HSV-1 behaves as a partially dominant trait. Although preferential loss of one parental set of chromosomes is not expected from these hybrids, it is clear that in some cases the average chromosome number in the hybrids is less than the sum of those of the two parents. Although we are unable to state conclusively that this was not due to preferential loss of chromosomes of one parent, there is no correlation between the extent of chromosome loss and susceptibility to HSV-1.

Hybrids were also constructed between a Don TK⁻ variant (a23) and human HGPRT⁻ skin fibroblasts from a Lesch-Nyhan patient (GM29). In this case the preferential and random loss of human chromosomes from such hybrid clones is well established¹⁹. A series of independently isolated Chinese hamster $\times$ human hybrid clones were assayed for HSV-1 susceptibility as before (Table 3). As can be seen, partial dominance of susceptibility was demonstrated by six of the hybrid clones, while the remaining seven clones were non-susceptible. The susceptible clones gave titres approximately 10^2 – 10^3 -fold less than that of the human parent, which is in agreement with the 10^2 – 10^3 -fold difference between Don $\times$ BHK hybrids and their BHK parent, whilst virus titres on the non-susceptible clones were similar to those obtained on Don. This result implies the existence of a partially dominant human chromosomal determinant for HSV-1 susceptibility which segregates out of the hybrids as human chromosomes are lost.

In order to determine the complement of human chromosomes

Table 4 Correlation between the presence of human chromosome 3 and HSV-1 susceptibility in Chinese hamster $\times$ human somatic cell hybrids

Chromosome 3	HSV-1 susceptibility	
	+	—
+	6	0
—	0	7

Giemsa-banded metaphase spreads of Chinese hamster $\times$ human hybrid clones were prepared as described previously²⁰. A minimum of 25 metaphase cells of each clone were scored for the presence of human chromosome 3; positive clones were those in which chromosome 3 was present in more than 50% of cells. The data on HSV-1 susceptibility were taken from Table 3. The table shows the number of clones found in each category.

in the hybrid clones, isozyme^{21,22} and karyotype analyses were performed. Using the technique of Cellogel electrophoresis the hybrids were analysed for the following isozyme markers: phosphoglucosyltransferase-1, isocitrate dehydrogenase, hexose-aminidase B, superoxide dismutase B, glutathione reductase, adenylate kinase-1, glutamic-oxaloacetic transaminase, lactate dehydrogenase A and B, nucleoside phosphorylase, pyruvate kinase, peptidase A, glucose phosphate isomerase, adenosine deaminase, superoxide dismutase A, and glucose-6-phosphate dehydrogenase. In no case was a correlation found between the presence of the human form of an enzyme in a hybrid and its susceptibility to HSV-1 infection, thus excluding by inference²³ a linkage to human chromosomes 1, 2, 5, 8, 9, 10, 11, 12, 14, 15, 18, 19, 20, 21 or X, respectively. Linkage to chromosome 17 is also excluded, since this chromosome carries the human thymidine kinase gene²⁴ which is required by all clones for survival in HAT medium.

Human chromosomes were readily distinguishable from those of the Chinese hamster in Giemsa-banded metaphase spreads of hybrid clones. Although extensive chromosome rearrangement was evident in one clone (PF4A1), in the majority of cases chromosome morphology was normal, and we were able to confirm the presence of those chromosomes whose presence was implied by the isozyme data. Of the remaining chromosomes only human chromosome 3 was present in all HSV-1 susceptible clones and absent from those which were non-susceptible. Although the total number of hybrid clones considered in this study is small the correlation shown in Table 4 is statistically significant ($\chi^2 = 6.20$; $0.01 < P < 0.05$), and we

therefore conclude that the gene(s) responsible for HSV-1 susceptibility in Chinese hamster $\times$ human hybrids is located on human chromosome 3. The nature of the host factor(s) specified by this gene must remain the subject of further investigations.

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Influence of multiple syngeneic foetal heart grafting on individual graft survival

THE factors which influence many developmental phenomena are poorly understood. One would like to know, for example, how it happens that an organism only develops one heart. A possible clue to this particular question arose inadvertently from an immunological tolerance assay involving inter-strain grafting of murine foetal hearts into adult ear pouches¹. We noticed amongst the control syngeneic grafts that whereas single heart grafts had a high long-term survival rate, grafting two hearts into a single animal seemed to considerably reduce the individual graft survival rate. The experiments reported here extensively document this observation using different strains and different grafting sites, as well as control grafts for specificity.

The recipient animals used in the study were highly inbred CBA/J and BALB/Ccr males between 2 and 4 months of age and were obtained from our breeding facility at the University of Alberta. No animal of a given strain was more than four generations removed from the ancestral breeding pair generating any other animal of that strain. Pregnancies were timed by checking for the presence of vaginal plugs, and the day after plugging was labelled day 1. All grafts reported in the study were obtained from 17-d foetuses. The principal method of grafting consisted of preparing a pouch under the dermis of the ear with the aid of small sharp scissors and tucking the graft as deeply as

possible into the pouch. Vascularisation of the foetal heart in this position permits recording of the graft's electrical activity simultaneously with that of the adult heart by specialised electrocardiography¹.

The results of a large number of such grafts performed in two different strains and read over the course of a 10-week period are shown in Table 1. The data for the CBA/J strain indicate that grafts put singly into an animal have a much higher probability of functioning than do grafts placed in an animal along with a similar graft in the opposite ear. The effect is reasonably stable over 10 weeks and the differences between the groups for any individual week are quite apparent. For example, even at week 1 the difference is highly significant ($P < 0.0001$) when tested by the chi-square contingency method. The results with a different strain of mice, BALB/Ccr, are concordant, although the overall functioning of both single and double heart grafts is somewhat reduced. The differences, although not as striking, are significant and the effect seems to disappear with time due to reduced functioning of the single heart grafts. In addition, a third strain (CBA/CaJ) was tested using 14 single grafts and 14 double grafts and the results at 2 weeks are similar to the previous data (57% functioning single hearts and 21% functioning double hearts, $P < 0.05$).

One possible explanation for the reduced survival of double heart grafts is that the added time and trauma involved in grafting the second heart somehow compromise both grafts. In order to test this, hearts were grafted into the left ears and either 17-d foetal kidney or 17-d foetal testis explants were grafted into the right ears of a group of CBA/J recipients. The hearts were checked at 2 weeks by electrocardiographic activity and then the ears were sectioned to examine the survival of the foetal kidney and testis grafts. Table 2 shows that neither foetal kidney nor foetal testis significantly affected the functioning of the grafted hearts at 2 weeks post-transplantation. The effect, therefore, seems to be specific for foetal heart. In order to further rule out artefacts that might ensue from the site of grafting and the method of reading, heart grafts were placed under the kidney capsule and scored one week later for beating, using an operating microscope. A heart was scored as positive if any fasciculation whatsoever could be detected within 1 min of viewing. The single hearts were implanted in the left kidney capsule and the double grafts were implanted either in the same kidney capsule or the contralateral kidney capsule. Since neither of these two situations differed in terms of overall frequencies, the results have been pooled. Table 3 shows that grafting under the kidney capsule gives a slightly higher proportion of beating compared to grafting in the ear, but the difference still exists between single and double grafts in two different strains. In fact the relative proportions of functional grafts for the two strains are in agreement by both methods of grafting and assessment (Tables 1 and 3). Grafting three hearts has no greater effect on an individual graft's functioning than grafting two hearts.

Since the adult heart does not interfere with single heart graft survival it is reasonable to enquire what effect an established heart graft would have on a second heart graft implantation. Therefore, we grafted single hearts in the left kidney capsule and 1 or 2 weeks later grafted a second heart in the right kidney capsule. The results of such a delay are shown in Table 4. The second graft seems to have no influence on the functioning of the first graft. The second graft, however, shows a significantly lower proportion of beating ($P < 0.05$). The proportion of second grafts functioning is higher than two hearts grafted at the same time, but not significantly different. This indicates that whatever is mediating this effect will not influence a graft that has been established for a week or two. An established

Table 1 Heart grafts in ear pouches

	1	CBA/J		3	4	Week 5	6	7	8	9	10
Fraction double heart grafts functioning	0.49	0.57	0.60	0.59	0.58	0.57	0.60	0.57	0.59	0.59	0.59
Total number	102	122	116	116	98	98	94	82	64	64	64
Fraction single heart grafts functioning	0.94	0.86	0.86	0.84	0.86	0.86	0.81	0.80	0.82	0.82	0.82
Total number	65	64	64	64	64	63	63	59	45	45	45
Significance of difference, <i>P</i> less than	10 ⁻⁴	10 ⁻⁴	10 ⁻³	10 ⁻³	10 ⁻³	10 ⁻³	5 × 10 ⁻³	10 ⁻²	2 × 10 ⁻²	2 × 10 ⁻²	2 × 10 ⁻²
		BALB/Ccr									
Fraction double heart grafts functioning	0.40	0.40	0.41	0.42	0.39	0.34	0.36	0.34	0.39	0.41	0.41
Total number	146	146	126	96	96	76	76	76	56	56	56
Fraction single heart grafts functioning	0.58	0.54	0.58	0.57	0.53	0.55	0.55	0.53	0.45	0.34	0.34
Total number	103	103	103	108	68	53	53	53	38	38	38
Significance of difference, <i>P</i> less than	10 ⁻²	3 × 10 ⁻²	2 × 10 ⁻²	5 × 10 ⁻²	NS*	3 × 10 ⁻²	5 × 10 ⁻²	5 × 10 ⁻²	NS*	NS*	NS*

NS = Not significant (*P* > 0.05).

graft, however, can have some effect on a subsequent graft.

The data presented in this paper support the conclusion that the probability of an individual heart graft surviving in a syngeneic host is influenced by whether or not other hearts are grafted at the same time. This conclusion has been reached after examining a large number of grafts in two different strains and a smaller number in a third strain. There are a number of possible explanations for this observation. The first and most obvious one, that of observer bias, seems to be ruled out because the grafts were read blind using the electrocardiographic procedure. The results were extraordinarily consistent from one reading to the next. In other words, if a given animal had a positive left ear and negative right ear, this persisted from week to week during the period for which it was read, even though there was no way in which the reader could have known the previous results while the ears were being read. Also, at various stages three different people read the grafts and came to the same conclusion.

A second possible explanation could come from the additional time involved in grafting the second heart, which might compromise both grafts. In fact, the heart grafting can be done so rapidly with a little practice (approximately 30 s per ear) that this objection is highly unlikely. Furthermore, no adverse effect of grafting other foetal tissues into a contralateral ear was observed on single heart grafts.

Another explanation that needs to be considered is an immunological one. A segregating or foetal specific antigen might cause some of the heart grafts to be rejected. Three separate considerations render this explanation untenable. First of all, the difference between double and single heart grafts is apparent after 1 week (see Table 1). This would be too rapid for most types of graft rejection. Secondly, histological examination of nine electrocardiographically negative hearts removed 2 months after ear grafting revealed normal heart muscle tissue, indistinguishable from control positive grafts, and showing no sign of monocytic or polymorphonuclear infiltration. Finally, and most conclusively, the difference between double-grafted and single-grafted hearts persisted when the host animals were completely immunosuppressed. This was accomplished by irradiating CBA/J males with 650 R using a ¹³⁷Cs source

(Gamma Cell 40, Atomic Energy of Canada), a dose which completely suppressed the splenic plaque forming cell response to sheep red blood cells when immunised 24 h and assayed 5 d after irradiation. (Six unirradiated control immunised animals had a response of 8.41 (±3.91) × 10⁴ PFC per spleen. Six normal unimmunised mice had 153 (±190) PFC per spleen. Six mice given 650 R and immunised had 65 (±45) PFC per spleen.) The mice had heart grafts placed in their ears 24 h after irradiation and read electrocardiographically 7 d after grafting. Of the double grafts, 57% (8/14) were positive at one week, whereas 92% (11/12) of the single grafts were positive. This result closely resembles the data in Table 1 and indicates that complete immunosuppression of the host does not increase the percentage of functioning double heart grafts.

A more attractive explanation of these results might be the existence of a mechanism whereby a nascent heart sends out some signal to prevent another heart from developing along the same vascular tree. This could have obvious relevance for survival if the probability of more than one inductive signal for heart formation is appreciable. This speculation agrees with the experiment in which an established graft can influence the functioning of a graft put in subsequently, but the subsequent graft has no effect on the established graft. If some such mechanism exists and operates by a soluble factor it would not be possible for the factor to cross the placenta (at least in the maternal to foetal direction) because mice invariably have multiple offspring in the same litter. The reason for the existence of some doubly positive animals might be that the relatively large "space" of the adult host in the experimental situation (compared to the ordinary foetal "space") somewhat dilutes the effect.

Analogous observations have been made with grafted neonatal lymphoid organs. Metcalf showed that single neonatal spleen grafts placed subcutaneously into adult splenectomised mice grew much larger individually than did

Table 2 Specificity of double heart effect

Group	Left ear	Right ear	Fraction beating*†	Total number
1	Heart	—	0.86	64
2	Heart	Heart	0.57	122
3	Heart	Kidney	0.81	16
4	Heart	Testis	0.83	18

*Read at 2 week post-grafting.

†Groups 3 and 4 are not significantly different from group 1 (*P* > 0.9) but are significantly different from group 2 (*P* < 0.01).

Table 3 Heart grafts under kidney capsule

Strain	Multiplicity of graft	Fraction beating*	Total number
CBA/J	1	0.93	29
CBA/J	2	0.64	28
CBA/J	3	0.62	21
BALB/Ccr	1	0.64	12
BALB/Ccr	2	0.50	11

*Read at 1 week post-grafting.

Table 4 Effect of established graft on second graft

	Fraction beating (total number) if second graft delayed for		
	1 week	2 weeks	Total
Established graft	1.00 (14)	0.93 (14)	0.96 (28)
Second graft	0.79 (14)	0.71 (14)	0.75 (28)

multiple grafts². In fact, the total spleen mass tended towards a constant value. Thus, six multiple grafts gave a total weight roughly equal to that observed with 12 multiple grafts. This effect was not observed when multiple spleen grafts were placed on the chorioallantoic membrane of chickens³. Metcalf found that neonatal thymus grafts, unlike spleen grafts, behaved autonomously, even though as many as 50 grafts were implanted subcutaneously, reaching a total weight of 800 g. Two different groups have reported nonetheless that the newborn offspring of neonatally-thymectomised females have enlarged thymuses compared to control litters born to sham-thymectomised females^{5,6}. The effect diminishes with subsequent pregnancies as the female herself becomes immunologically restored^{6,7}. It is not inconceivable, therefore, that a similar type of feedback mechanism operates during the development of the heart to prevent the disastrous consequences of two autonomous pumps operating on the same vascular tree.

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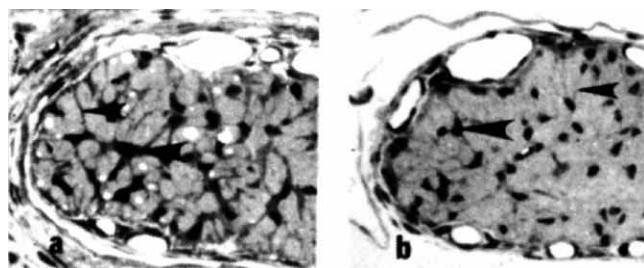


Fig. 1 Transverse sections of optic nerves from a 2-d postnatal Jimpy mouse (a) and a hemizygote (b). Both pictures are at the same magnification ($\times 150$) and they illustrate differences in the size of nuclei (large arrows) and the hypertrophy of astroglial processes (small arrows). The degree of the hypertrophy in Jimpy optic nerves is variable from animal to animal and even within the nerve. Note that the left side seems more affected than the right. The vacuoles are not artefacts of fixation but seem to be swollen, degenerating axons at the fine structural level.

these animals showed probable glial and axonal abnormalities at the fine structural level. Accordingly, homozygous normals and Swiss mice were also used.

During early postnatal development in the optic nerves of normal mice and rats, astrocytic processes divide the neurites into distinct fascicles⁷⁻⁹. These processes do not have many side branches but in Jimpy many fine branches penetrate into the axonal fascicles isolating neurites from each other (Fig. 2). This hyperplasia of astrocytic processes continues during myelinogenesis^{10,11} until the majority of axons are surrounded (Fig. 3).

In normal animals at 2 d after birth, the cytoplasm of astroglial cells contains an abundance of organelles including microtubules; the clusters of filaments characteristic of mature forms are less common⁷⁻⁹. In Jimpy mice at 2 d after birth, this relationship is reversed so that there is a paucity of microtubules and an abundance of filaments (Table 1). There is almost a fivefold difference in the number of microtubules in Jimpy when compared to Swiss

Myelin deficit in the Jimpy mouse may be due to cellular abnormalities in astroglia

THE mouse mutant Jimpy is considered to be a model for certain human diseases in which the development of myelin is abnormal^{1,2}. Biochemical studies of Jimpy have shown that myelin components are reduced but no specific abnormality in the assembly of myelin has been detected²⁻⁴. This electron microscopic study describes for the first time abnormalities in astroglia of Jimpy optic nerve that are likely to inhibit the development of oligodendroglial cells and myelination.

Before the onset of oligodendrocyte formation in these mutants, processes of astrocytes begin to branch abnormally. The branching continues until most axons are surrounded by astrocytic processes. Such an arrangement would make it necessary for the oligodendrocytes to displace the glial processes in order to contact axons and make compact myelin. The cause of the astrocytic branching in Jimpy has not been proven but it may be related to the paucity of microtubules and profusion of 90-Å filaments found in astrocytes during the early stages of development. Microtubules are necessary for maintaining cell shape^{5,6}, so it is possible that their paucity and/or the filamentous hyperplasia is the underlying morphological defect responsible for the shape of astroglia in Jimpy.

Jimpy mice, obtained from breeding colonies sent by Jackson Laboratories, were studied at 2, 5, 10 and 23 d after birth. Optic nerves were examined at all stages and compared with spinal cords at 2 and 23 d after birth. Female hemizygotes were initially used as the only controls but

Fig. 2 Electron micrograph of the optic nerve from a 2-d-old Jimpy animal showing the branching of astrocytic processes (Ap) into fascicles of axons (A). The large, radially oriented processes give off numerous small branches which divide the axons into small compartments. Some of these processes have begun to separate individual axons from other axons (arrows). This extensive branching does not occur in normal animals (see references 7-9 for comparison illustrations). The radially oriented astroglial processes contain primarily 90-100-Å filaments but few microtubules (Table 1) ($\times 8,800$).

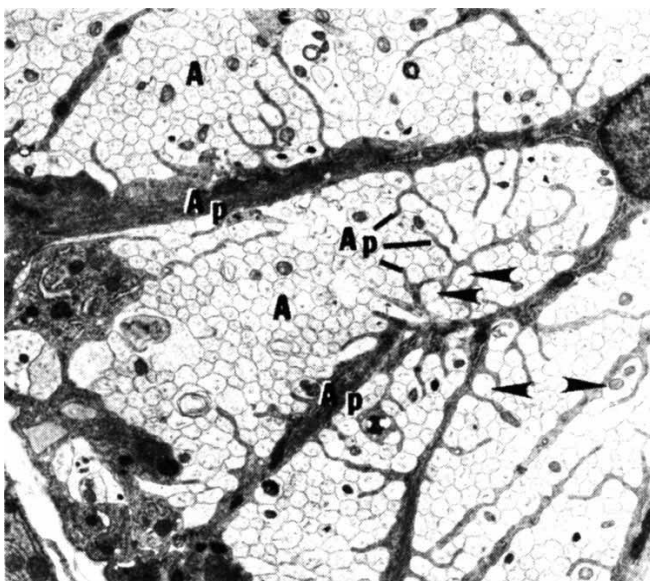


Table 1 Comparison of number of microtubules and filaments in astroglia of 2-d postnatal optic nerve

	Swiss strain	Hemizygote	Jimpy
No. of astrocyte microtubules per μm^2 of astrocyte cytoplasm	34.3	16.0	7.2
No. of astrocyte microtubules per μm^2 of optic nerve tissue	7.5	4.3	2.2
No. of astrocyte 90-Å filaments per μm^2 of astrocyte cytoplasm	~480*	~640	~1170
Ratio of astrocyte cytoplasm to optic nerve tissue	1:4.6	1:3.9	1:3.3

The number of longitudinally sectioned microtubules in astroglia was counted from electron micrographs printed at 13,000 and viewed with a Bausch and Lomb $\times 7$ magnifier. Ten to fifteen random pictures were used for each optic nerve; half of the pictures were taken from the centre of the nerve and the other half at the periphery. The area of the astrocyte cytoplasm was calculated with a Lasico planimeter. The area of optic nerve tissue included all cell nuclei and processes with the exception of vascular elements. The area of astrocyte cytoplasm surveyed ranged from 6.5 to 7.3 μm^2 . The quality of fixation for all animals was approximately the same.

* The precise number of 90-Å filaments is impossible to determine because the filaments overlap each other and weave in and out of the plane of the picture. The estimate for Jimpy is probably low because of the dense packing of filaments within a cluster.

mice and a twofold difference for the hemizygotes. It might be argued that the microtubule deficit is only an apparent one because the astrocytic cytoplasm is hypertrophied. But when the number of microtubules in astroglia is compared to the area of optic nerve (Table 1), there is still a two- to fourfold difference between Jimpy and the controls. The difference in the ratios between astrocytic cytoplasm and optic nerve area confirms the light and electron microscopic observations that there is a hypertrophy of astrocyte cytoplasm (Fig. 1). In contrast to the microtubule deficit, the number of 90-Å astroglial filaments is increased more than twofold (Table 1). Electron microscopic observations show that not only are the clusters of filaments more numerous but also that the filaments within the clusters are more densely packed. This observation correlates well with an immunofluorescent study¹² of astroglial fibrillary acidic protein in Jimpy mice. Although not commented on by these authors, the fluorescence for this substance is greater in Jimpy spinal cords than in the littermates used as controls (Fig. 1). This study shows that microtubule and filament abnormalities occur to a lesser degree in the hemizygotes.

While the astroglial cells in Jimpy optic nerve are strikingly abnormal in appearance, most of the oligodendroglia seem normal except that they are less differentiated than the controls. The number of oligodendroglial cells is, however, reduced more than 50% (Table 2). The percentage reduction of oligodendroglia depends, to some extent, on the interpretation of the unclassifiable cells. If all of the unclassifiable cells are considered to be oligodendroglia, there is a 48% reduction at 10 and 23 d after birth; if none is considered oligodendroglia, the loss is 61% and 79%. But approximately half of the unclassified cells at 10 and 23 d after birth seem to be oligodendrocytes and using this estimate, there is about a 53% and 62% reduction. An apparent reduction in the number of oligodendroglia in the corpus callosum has been noted^{13,14} but the severity of this deficit was not quantitated.

In previous studies of Jimpy nervous system, investigators have focused upon the period of myelin formation, and have primarily confined themselves to possible oligodendroglial and myelin abnormalities^{2-4,10,13-15}. The hyperplasia of astroglial processes described here for the optic nerve is, however, frequently noticeable in the published pictures (ref. 15, Figs 7 and 8). We show that these abnormalities are apparent at least a day before oligodendroglia are present and 3 or 4 d before myelination begins^{10,11}. This observation indicates that the astroglial hyperplasia is not triggered by the presence of oligodendrocytes.

The astroglial abnormalities raise the interesting question as to how they can affect the oligodendroglial cells. It is conceivable that the hyperplasia of astroglial processes, which occurs before and during myelinogenesis, could block the oligodendrocytic processes from reaching axons and forming proper internodes. However, it is more difficult to explain how the proliferation of oligodendrocytes can be

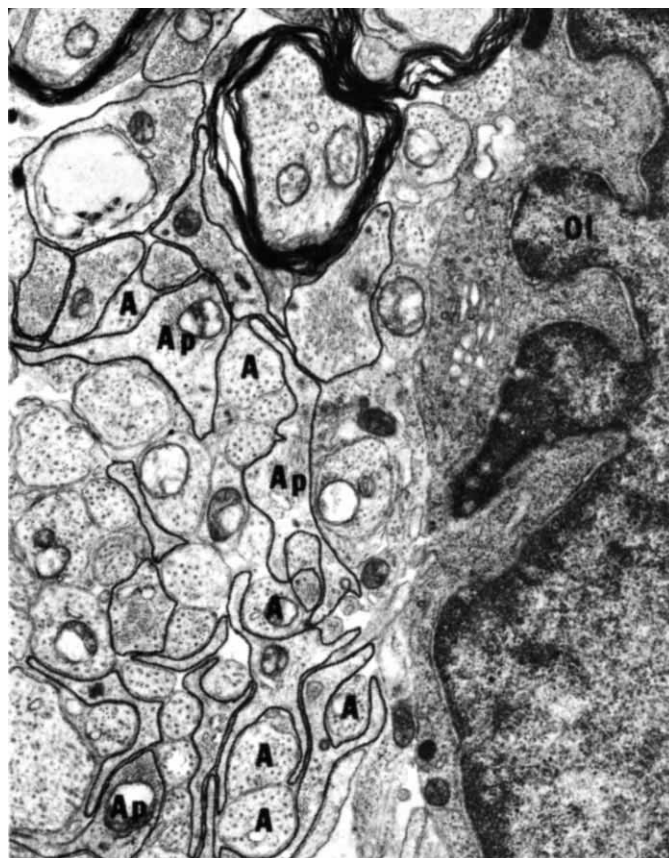


Fig. 3 Electron micrograph of a Jimpy optic nerve 23 d after birth. The astrocytic processes (Ap), most of which have been outlined, now surround the majority of axons (A). The glial processes are identified by the presence of numerous 90–100 Å filaments which appear as clusters of dots at this magnification. The profile of an oligodendrocyte (Ol) is identified by its electron dense cytoplasm and nucleus. The clumping of chromatin beneath the nuclear membrane is also a characteristic feature of this cell type. Three axons are surrounded by four to six turns of myelin lamellae ($\times 14,500$).

affected. Although the factors controlling oligodendroglial proliferation are unknown, the proliferation of the myelin-forming cells in the peripheral nervous system is stimulated by the presence of axons¹⁶. Perhaps the astroglial hyperplasia impedes contact between the axons and oligodendrocytes. Another possibility is that the normal number of oligodendroglial cells is generated but that some die shortly after cell division, due to lack of contact with axons. Thymidine autoradiographic studies should provide information about the proliferation kinetics of these cells. A third possibility is that the source of the oligodendrocytes might be affected. The origin of the oligodendroblast stem cell is

Table 2 Ultrastructural classification of different cell types present in a complete transverse section of optic nerve.

	Astroglia	Oligodendroglia	Microglia	Unclassifiable	Total cell no.
2-d postnatal Swiss mouse	54	0	0	19	73
2-d postnatal hemizygote	57	0	0	15	72
2-d postnatal Jimpy*	61	0	2	3	66
10-d postnatal homozygous normal	70	81	1	28	180
10-d postnatal Jimpy	75	32	1	25	133
23-d postnatal homozygous normal†	59	97	4	12	172
23-d postnatal Jimpy	60	20	8	37	125

The ultrastructural criteria for classification of differentiating glia in optic nerve is discussed in detail by Skoff *et al.*⁷ In the present study, two characteristic features of each cell type were required for a definitive classification (for example, filaments and electronlucent appearance for astrocytes). It is difficult to classify precisely all of the cells because some do not have sufficient characteristics for a positive identification or they have been sectioned through areas lacking cytoplasm. Complete transverse sections of the optic nerve were mounted on Formvar coated slot grids for electron microscopic examination. The cell counts were made directly from the viewing screen of an AEI-801 electron microscope. A scanning system was devised to avoid recounting the same cell; low magnification montages were made of two sections to check possible errors in counting. There was a 1 and 3% difference in the number of labelled cells using the scanning and montage methods. Only one animal was used for quantitation but at least two Jimpy animals were examined at each stage except at 23 d after birth when only one animal was available. The procedure for preparing optic nerve tissues for electron microscopy has been presented elsewhere⁷.

*2 d postnatal animals were identified as Jimpy on the basis of differences in the amount of myelin present in the spinal cord. At this time, axons in the ventral funiculus are beginning to myelinate and the spinal cords of Jimpy animals show fewer myelinated axons.

†The number of cells counted in a transverse section of a homozygous normal (172) compares favourably with a light microscopic cell count (162) by Friedrich¹⁸ of a (C57BL/6J) mouse.

uncertain but there are two likely sources⁷, a population of undifferentiated cells or the poorly differentiated astroblasts. The number of undifferentiated cells and poorly differentiated astroblasts is reduced in Jimpy optic nerve 2 d after birth, in comparison to controls.

Previous morphological and biochemical studies of Jimpy mice have shown that the scarcity of myelin is restricted to the central nervous system¹ and that the deficit is not due to a demyelinating disorder^{2,15}. Up to the present, however, no specific abnormality in the formation of myelin has been detected by biochemical studies²⁻⁴. The reduction in the number of oligodendrocytes described here is certainly one of the major factors responsible for the paucity of myelin in Jimpy but it does not elucidate the cause of the cellular reduction nor does it explain certain abnormalities in the appearance of the few myelinated fibres. The hyperplasia of astrocytic processes, which begins before the formation of oligodendroglia, could account for the myelin and oligodendroglial abnormalities. The hypothesis that astroglia affect oligodendrocytes is new but a precedent for such a cellular interaction in the developing central nervous system occurs between astrocytes and neurones in which the migrating neuroblasts fail to reach their destination in the absence of astroglial processes¹⁷. The effect of an overproduction of astrocytic processes on neuronal development has yet to be determined but the precocity of the "gliosis" in Jimpy, occurring when axons are still growing through the optic nerve, may be a model for studying the interactions between astroglia and axons in development.

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Reversible loss of hippocampal long term potentiation following electroconvulsive seizures

ONE of the most intriguing electrophysiological phenomena observed in the central nervous system is the persistent enhancement of an evoked synaptic response following brief trains of electrical stimuli. Within the hippocampus, evoked synaptic potentials can be doubled or tripled by tetanising stimulation, and such changes persist for hours to weeks¹⁻⁶. Interest in the phenomenon of long term potentiation (LTP) stems from the generally held belief that synaptic plasticity of this sort may well be a physiological basis of information storage in the CNS. It is notable that this striking synaptic plasticity is observed in a structure which extensive clinical and experimental evidence suggests has a central role in information storage and/or retrieval⁷⁻¹¹. Included in this evidence are numerous demonstrations that disrupting hippocampal activity by electrically induced epileptiform seizures also disrupts memory formation and/or retrieval¹²⁻¹⁵. In addition, the effectiveness of a variety of other treatments in disrupting behavioural plasticity seems closely related to their ability to elicit convulsive activity in the hippocampus^{16,17}. We report here a series of experiments indicating that LTP established in the hippocampus can also be reversibly disrupted by the same kind of convulsive activity which disrupts memory functions. Specifically, we have produced LTP in the CA1 pyramidal cell synaptic field, disrupted it with electrically induced hippocampal seizures, and subsequently reinstated it with low frequency tetanic stimulation.

Adult Lashley rats were given atropine sulphate (intramuscularly, 0.2 mg kg⁻¹) and anaesthetised with an intraperitoneal injection of allobarbitol (60 mg kg⁻¹) and urethane (240 mg kg⁻¹). The hippocampus was exposed by aspirating the overlying cortex, and was continuously bathed in warm (37-38 °C) mineral oil. Electrodes were placed to stimulate fibres within the stratum radiatum of the CA3 field and to record the extracellular population excitatory postsynaptic potential (e.p.s.p.) from stratum radiatum of the CA1 field. An additional electrode, well removed from the other two, was used to generate electroconvulsive

seizures in the hippocampus. In most cases an input-output function was first obtained by recording the CA1 synaptic response to CA3 stimulation over a range of stimulus intensities. Subsequently, several low frequency trains of impulses (12 Hz for 15 s, 8 min inter-train interval) were delivered while monitoring the CA1 synaptic response with a probe stimulus continually applied at 0.2 Hz.

The low frequency tetanic (LFT) stimulation produced long term increases in the evoked population e.p.s.p. amplitude ranging up to about 300% (mean 175.8%), along with decreases in the stimulus intensity required to elicit a population spike from the CA1 pyramidal cells. The effects of LFT stimulation were cumulative, and maximum potentiation was usually obtained after three to six LFT trains. That the potentiation was truly long term was established in several experiments where it persisted with little or no decline for periods of over an hour (the duration of the experiment) following the last LFT.

In 18 experiments hippocampal seizures were induced at various intervals following a series of LFT trains. Seizures were evidenced by afterdischarges lasting up to 45 s, followed by complete post-ictal depression of the response for up to 10 min, then a period of gradual response recovery. Recovery was considered complete when the response showed no further change for 10 min. The overall recovery period ranged from 15 to 25 min following the seizure.

The effect of these induced seizures was dramatic (Fig. 1). In all 18 experiments the LTP established by repeated tetanic stimulation was wholly or partially abolished. In none of these experiments did the post-seizure response recover to the potentiated response level, and in all but two cases the post-seizure response recovered to at least the level of the initial unpotentiated control response. In both of these cases a sudden drop in the response during the re-

covery period suggested probable electrode movement. The magnitude of the drop was approximately equal to the difference between the final recovery and initial control responses. Excluding these two experiments, the effect of the seizures was to reduce the LFT-induced response potentiation by a mean 83.5% (the mean post-recovery response was 119.8% of the initial control response).

The interference with LTP produced by the seizure seemed partially or wholly reversible. In ten experiments additional LFT trains were administered after a seizure had wholly or partially eliminated the LTP established by the initial LFT stimulation. In eight of these experiments LTP was re-established, and such plasticity was evident through as many as four cycles of the LFT-seizure-recovery procedure (Fig. 2).

As is evident in both Figs 1 and 2 there was, in general, a progressive decrease in the amount of LTP which could be realised following successive seizures. In those experiments in which LFT was administered after a seizure, the second LFT series produced only about half (45.2%) as much plasticity as the pre-seizure stimulation produced. Comparable decreases in plasticity seemed to be the rule following a second or third seizure. It is not clear whether the decreasing potential for plasticity represents a real limitation of the processes involved or is simply a "tired tissue" phenomenon. The latter possibility seems probable, since it is noted that less electrophysiological plasticity of any type is evident in longer experiments⁵.

These results might be interpreted as being due to an inadequate period of recovery following the seizure. To test this possibility, post-seizure responses were monitored in several experiments for 20–50 min after stabilisation rather than the usual 10 min (Fig. 2c). Despite this, no additional recovery was noted. Hence, these results cannot

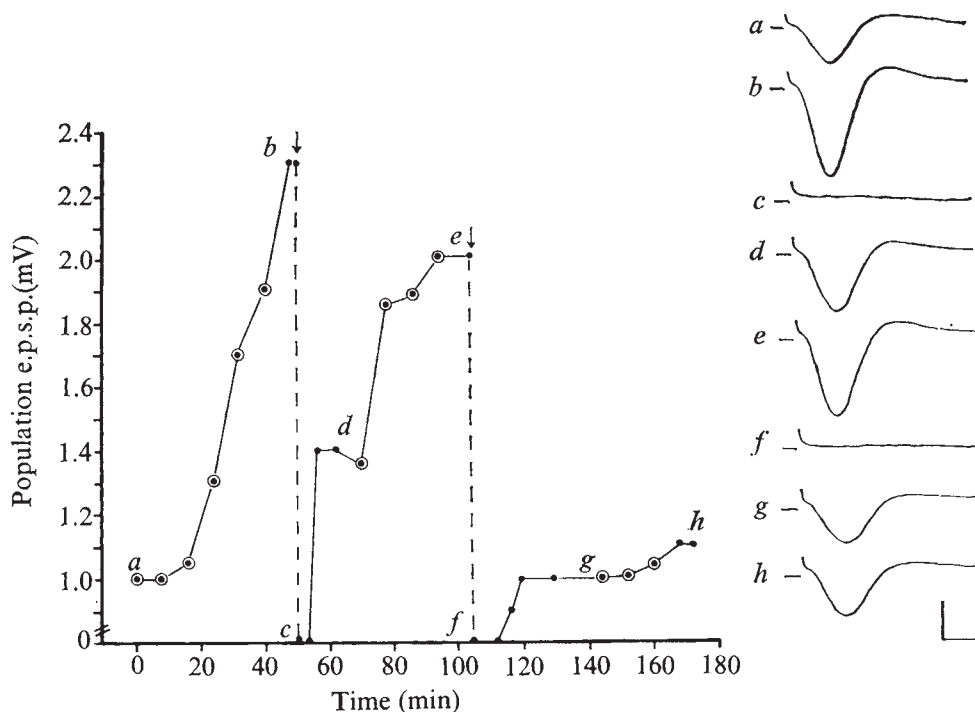


Fig. 1 CA1 extracellular population e.p.s.p. response magnitude to test stimuli during the establishment of long term potentiation (circled points), its interruption by electrically induced electroconvulsive seizure (arrows), and recovery from seizure related post-ictal depression (following the dashed lines) in a typical experiment. The circled points are test responses immediately preceding low frequency tetanic stimulation (12 Hz for 15 s). The low frequency tetanic stimulation resulted in increases in synaptic efficacy which were abolished by the seizure. Following recovery from post-ictal depression, additional tetani again resulted in a potentiated response, but of progressively lower magnitude. Raw records are shown corresponding to the indicated points on the graph. CA1: 1 mV, 5 ms. Control (0.2 Hz, 0.1 ms duration, 5–20 V) and tetanic (12 Hz, 0.1 ms duration, 5–20 V) stimulus pulses were delivered through an insulated monopolar stainless steel electrode (20–40 μ m exposed tip) placed in stratum radiatum of CA3 to activate axons (primarily Schaffer collaterals) projecting to CA1. A similar monopolar recording electrode was placed in stratum radiatum of CA1 (about 4 mm posterior to the stimulating electrode) to record the CA1 extracellular population e.p.s.p. A similar monopolar stimulating electrode was placed 1 mm deep posterior and lateral to the recording site. Seizures were induced through this electrode by a 1-s train of 60 Hz, 15 V pulses (biphasic, 1 ms duration, roughly 300–400 μ A).

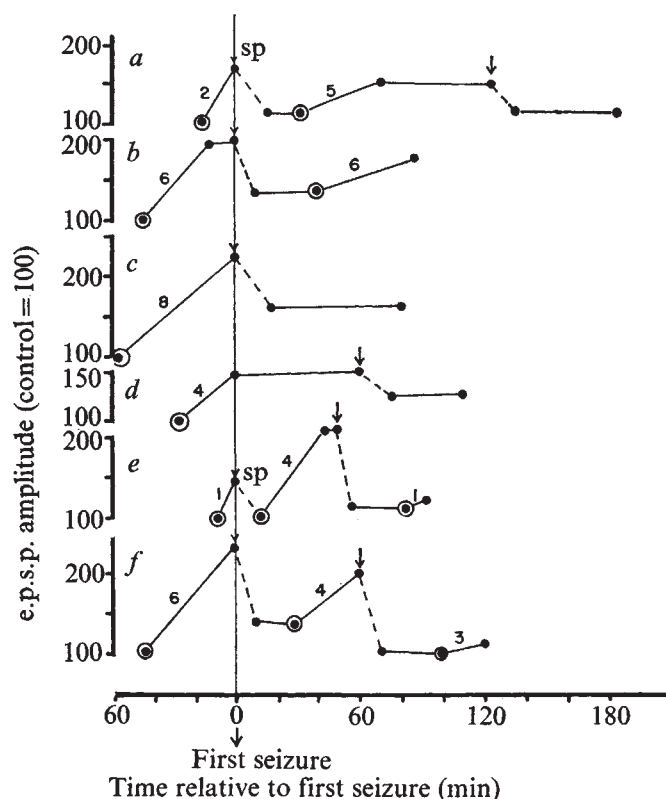


Fig. 2 Six experiments (a-f) in which a number of variables were manipulated relative to seizure disruption of long term potentiation. Circled points are extracellular population e.p.s.p. magnitudes plotted relative to a pre-tetanus control (= 100). The numbers refer to the number of tetanic stimulations delivered; the following point is the stimulation induced potentiation. Electrically-induced or spontaneous (sp) seizures are indicated by arrows. The first seizure occurs at time zero (except for d). Note that the seizure interferes with long term potentiation regardless of the number of tetani, time after initial potentiation, or means of inducing seizures. Additional tetani result in repotentialization.

be readily attributed to inadequate recovery from seizure.

Another alternative explanation for these results is that the electroconvulsive stimulation and seizures produced a generalised decrease in the excitability of the hippocampus. That this was not the case is evidenced by several types of data. Seizure and recovery prior to LFT stimulation did not alter the response to the probe stimulus, nor did this preclude subsequent establishment of LTP. In some experiments where no LTP was observed, a seizure was nevertheless induced following several ineffective LFT trains. In these cases the post-recovery response was virtually unchanged from the pre-seizure level. In addition, in only two experiments (those noted earlier) was the threshold stimulation required to elicit a population e.p.s.p. appreciably different following seizure recovery than the threshold before the seizure, and before the LFT stimulation^{5,6}. It should also be noted that in most cases pre-seizure LFT stimulation was continued until no further response increases were observed, and the response was "fully potentiated." In spite of this, additional plasticity was evident following a seizure. Finally, in three cases "spontaneous" seizures occurred during an experiment with the same result as electrically-induced seizures—total or partial elimination of LTP (Fig. 2a, e).

The data presented here indicate that electroconvulsive seizures induced in the hippocampus interfere with the processes underlying long term potentiation of evoked synaptic responses, and that this interference is at least partially, and perhaps wholly, reversible. Thus, hippocampal seizures seem to have quite analogous effects on both behavioural and electrophysiological plasticity. The evidence presented

here does not allow a decision on the relative importance of the convulsive activity *per se* against that of the post-ictal spreading depression (SD) in producing these effects. However, preliminary observations of a similar effect following topical application of KCl suggest that SD is the essential correlate of LTP interference.

We cannot comment conclusively on mechanisms which might mediate the effects described here, since the mechanisms underlying LTP are as yet unclear. Nor do we know if processes similar to these occur as a normal concomitant of hippocampal functioning. But our results seem consistent with one proposed mechanism of LTP. Van Harreveld and Fikova¹⁸ have proposed that LTP is the result of stimulation-induced swelling, and consequent conduction increase, of dendritic spines. Anatomical evidence seems to support their assertion, and there is limited evidence of the possible behavioural relevance of this mechanism^{19,20}. It is also notable that SD is accompanied by dendritic and somatic swelling, presumably mediated by the same mechanism responsible for stimulation-induced spine swelling, though in a more extreme manifestation. Since SD swelling is clearly reversible²¹, it is plausible to suggest that the extensive SD induced by seizures in our experiments triggered recovery processes which reversed not only dendritic and somatic swelling, but the presumed LFT-induced spine swelling as well. Thus, recovery from a seizure would be accompanied by a reduction or elimination of any LTP existing prior to the seizure, but would not preclude the re-establishment of LTP by further stimulation. Though this is clearly only a tentative hypothesis, it is consistent with the available data, and it does suggest additional experiments which could decide these issues.

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Evidence for dopamine receptors mediating sedation in the mouse brain

APOMORPHINE, a direct stimulant of dopamine (DA) receptors, and L-dopa, the direct precursor of DA, have a biphasic action on behaviour; in low doses they decrease motor activity, while in higher doses they cause hypermotility and stereotypy¹⁻³.

While the stimulant effect is considered to be due to the activation of postsynaptic DA receptors in brain^{4,5}, it has been suggested that the depressant response to these drugs is due to the activation of DA "autoreceptors", which would

result in inhibition of DA synthesis and dopaminergic firing^{3,6,7}.

However, while there is experimental evidence that the inhibition of DA synthesis and neuronal firing, which is blocked by neuroleptics, is due to stimulation of DA receptors^{6,8}, no such evidence exists for the sedative effect. Moreover the idea that the sedative effect of apomorphine depends on decreased dopaminergic activity is simply based on the observation that the two events are correlated in dose-response studies².

The demonstration that the sedative response to apomorphine and L-dopa is due to stimulation of DA autoreceptors is hampered by the fact that neuroleptics, which are antagonists at CNS DA receptors, themselves cause sedation.

We considered that some neuroleptic compounds might be found with higher affinity for auto- than for postsynaptic receptors and that such compounds might thus demonstrate the DA-mimetic nature of the sedative effect of DA receptor agonists.

We report here that some neuroleptics, at doses lower than those producing sedation, are capable of preventing the sedative effect of apomorphine and L-dopa in mice. At these doses these compounds are also effective in preventing the apomorphine-induced decrease in DA synthesis, as reflected by brain DA metabolite levels.

Figure 1 shows the effect of 0.1 mg kg⁻¹ of subcutaneous apomorphine in control mice and in mice pretreated with pimozide at the non-sedative dose of 0.3 mg kg⁻¹, 20 min previously.

Apomorphine decreased motor activity, and this effect

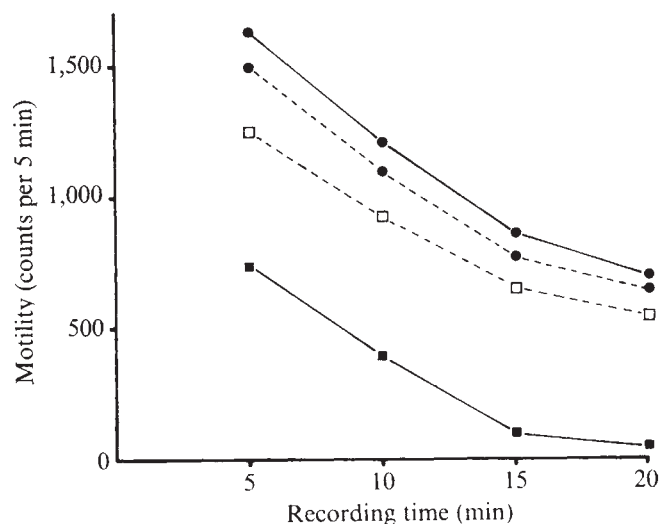


Fig. 1 Effect of pimozide on the hypomotility produced by apomorphine in mice. Mice were pretreated with pimozide (0.3 mg kg⁻¹ intraperitoneally) 20 min before apomorphine (0.1 mg kg⁻¹ subcutaneously). Ten min after apomorphine the animals were placed for the first time in the motility cage (Motron, Sweden) and motility was measured immediately. In these conditions, this generally took the form of exploratory activity. At the dose used in this experiment, apomorphine dramatically reduced the activity such that after the first 10 min, while control mice were still highly active, mice treated with apomorphine lay down in a corner and appeared sedated. The schedule used for pimozide was the most effective in preventing the sedation produced by apomorphine without producing sedation *per se*. Use of longer time intervals (3 h) for pretreatment with the 0.3 mg kg⁻¹ dose of pimozide results in sedation. However, similar antagonism of apomorphine hypomotility, even if less dramatic, was obtained after a 3-h pretreatment with 0.1 mg kg⁻¹ of pimozide. The results are the mean of four experiments. Open symbols indicate a significant difference ($P < 0.01$) between the curves with the same symbols as evaluated by non-parametric multivariate analysis¹⁹. ■, apomorphine; □, pimozide and apomorphine; ○, pimozide; ●, control.

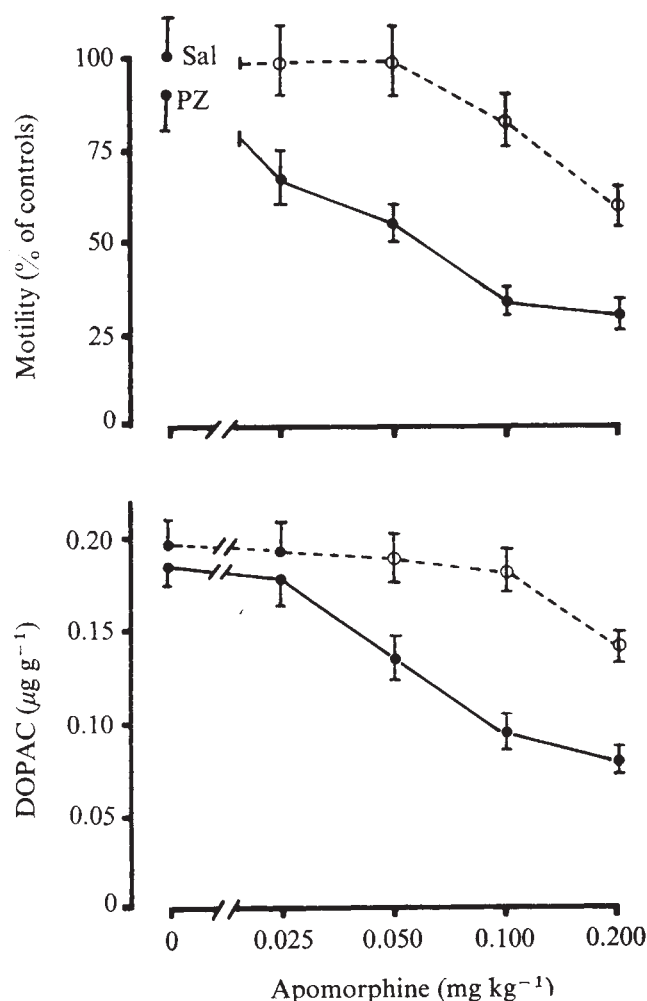


Fig. 2 Antagonism by pimozide of the hypomotility and decrease of brain DOPAC produced by apomorphine in mice. Pimozide (0.3 mg kg⁻¹ intraperitoneally) was administered 20 min before apomorphine. Apomorphine was administered subcutaneously and 10 min later the animals (4 cage) were placed in the motility meters (Motron, Sweden), and motility counted for 20 min. Mice were killed at the end of the motility test and DOPAC assayed fluorometrically according to Di Chiara *et al.* (unpublished). The effect of pimozide on apomorphine hypomotility was calculated as % of the motility obtained after pimozide alone. The results are the mean $\pm$ s.e.m. of 4 experiments. Open circles indicate a significant difference ($P < 0.01$) with respect to apomorphine alone. ●, apomorphine; ○, pimozide and apomorphine.

was prevented by pimozide; the latter also prevented apomorphine-induced decrease of brain 3,4-dihydroxyphenyl acetic acid (DOPAC), the deaminated metabolite of DA.

Figure 2 shows the effect of increasing doses of apomorphine on the motor activity and brain DOPAC of control mice and of mice pretreated with pimozide, 0.3 mg kg⁻¹, given 20 min earlier. Apomorphine caused a dose-dependent decrease in motor activity and of DOPAC levels both in control and in pimozide-treated mice. However, pimozide, which *per se* did not significantly influence motor activity or brain DOPAC, shifted to the right the dose-response curve of apomorphine-induced hypomotility and brain DOPAC decrease, suggestive of competitive antagonism.

The antagonism exerted by pimozide on the effects of apomorphine was not related to a modification of the tissue distribution of apomorphine, since pimozide did not modify the time-course of the levels of ³H-apomorphine in brain (results not shown).

Similar results were obtained with other neuroleptics such as haloperidol, benzperidol, droperidol and sulpiride: as shown in Table 1, these compounds, at doses which did not

influence motor activity or brain DOPAC, prevented both the hypomotility and the fall in brain DOPAC produced by apomorphine.

On the contrary, clozapine and trifluoperidol, at non-sedative doses, failed to influence either the sedative or the biochemical effects of apomorphine.

Neuroleptics active against the inhibitory effects of apomorphine were also able to prevent the sedative effect of L-dopa, when administered at a schedule similar to that used against apomorphine. As Fig. 3 shows, sulpiride shifted to the right the dose-response curve of L-dopa-induced hypomotility, again suggesting a competitive-type antagonism, as for pimoizide against apomorphine.

In the above conditions, sulpiride did not influence the amount of DA formed in brain after L-dopa administration.

The present results show that different neuroleptics, such as haloperidol, droperidol, pimoizide, benzperidol and sulpiride are able to prevent the sedative effect of apomorphine and L-dopa and also the ability of apomorphine to inhibit the activity of the DA system, as estimated by the decrease in brain DOPAC^{9,10}. Since neuroleptics are specific and competitive antagonists of the DA receptors both *in vivo* and *in vitro*¹¹⁻¹³, the present results strongly indicate that apomorphine and L-dopa produce sedation and decrease dopaminergic activity by stimulating central DA receptors, as suggested by Carlsson and by Strömbom^{2,3}. Moreover, the strict correlation between the behavioural and biochemical changes indicate that the sedative effect of apomorphine depends on the decreased DA activity.

As to the nature of the DA receptors mediating the inhibitory effects of L-dopa and apomorphine, they should be considered quite distinct from the inhibitory receptors

Table 1 Effect of various neuroleptics on the hypomotility and brain DOPAC decrease produced by apomorphine

Neuroleptic	Dose (mg kg ⁻¹)	Motility (% of control)	DOPAC (µg g ⁻¹)
Control	—	100 ± 11	0.185 ± 0.008
None	—	25 ± 3.2	0.095 ± 0.006
Benzperidol	0.050	85 ± 9.5	0.180 ± 0.010
Droperidol	0.050	80 ± 7.5	0.178 ± 0.009
Haloperidol	0.050	75 ± 8.5	0.175 ± 0.008
Pimoizide	0.300	83 ± 9.3	0.182 ± 0.009
Sulpiride	10.0	87 ± 8.5	0.192 ± 0.010
Trifluoperidol	0.050	27 ± 3.0	0.098 ± 0.007
Clozapine	0.50	23 ± 2.5	0.097 ± 0.006

All neuroleptics were administered intraperitoneally 20 min before apomorphine (0.1 mg kg⁻¹ subcutaneously); mice were placed in the motility cages (4 mice per cage) 10 min after apomorphine administration and motility counted for 20 min. At the end of the motility test, the animals were killed and DOPAC assayed fluorimetrically according to Di Chiara *et al.* (in preparation). The dose of neuroleptic reported in the table is the lowest non-sedative one which was found experimentally to be maximally effective in antagonising apomorphine hypomotility. In the case of trifluoperidol and clozapine, for which antagonism could not be shown, the dose reported is the highest non-sedative one. At the dose reported, neuroleptics alone did not modify significantly the control levels of brain DOPAC nor the motility. Nonetheless, the effect of neuroleptics on apomorphine hypomotility was always calculated as % of the motility obtained when the neuroleptics were administered alone.

Each value is the mean ± s.e.m. of 4 experiments. All neuroleptics, except trifluoperidol and clozapine, significantly ($P < 0.01$) modified motility and brain DOPAC with respect to apomorphine alone.

postulated by Cools *et al.*¹⁴, which are not stimulated by apomorphine and are not blocked by neuroleptics. Our results support the concept of regulatory DA receptors by which the DA system controls its own activity (autoreceptors)³. The exact location of these receptors is not clear. They might be prejunctional, that is, located on the membrane of the DA neurone itself and stimulated by an overflow of the transmitter^{8,15}, or in the substantia nigra, where a postsynaptic DA-sensitive adenylate cyclase^{16,17} and a vesicular release of DA from dendrites have been shown¹⁸.

The results reported here indicate the possibility of influencing behaviour by acting with drugs on a special type of DA receptor; this might constitute the basis for the development of more potent and selective blockers of DA autoreceptors and might provide an entirely new means of controlling the activity of the DA system.

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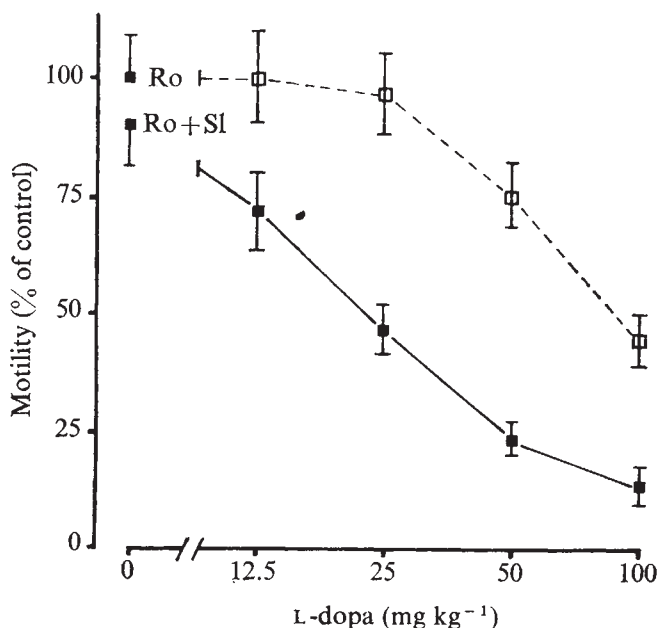
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Fig. 3 Antagonism by sulpiride of the hypomotility produced by L-dopa in mice. Mice were pretreated with 10 mg kg⁻¹ intraperitoneally of sulpiride, and 10 min later various doses of L-dopa were administered intraperitoneally together with a standard dose of the decarboxylase inhibitor Ro 4-4602/1 (50 mg kg⁻¹). Control mice received Ro 4-4602/1 alone or after sulpiride. Mice were placed in the motility cages 30 min after L-dopa+Ro 4-4602/1 and motility counted for 20 min. The effect of sulpiride on L-dopa hypomotility was calculated as % of the motility obtained after sulpiride+Ro 4-4602/1. The results are the mean ± s.e.m. of 4 experiments. Open symbols indicate a significant difference ($P < 0.01$) in respect to L-dopa+Ro 4-4602/1. □, sulpiride+L-dopa+Ro; ■, Ro+L-dopa.



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β -Endorphin *in vitro* inhibition of striatal dopamine release

THERE has been considerable recent interest in endogenous peptides as possible analgesic agents since the isolation of a pentapeptide with opiate-like activity (methionine-enkephalin) from porcine brain¹. Of particular interest is β -endorphin, isolated from camel pituitary glands², and now synthesised³. We report here the inhibitory effect *in vitro* of β -endorphin on striatal dopamine release from the central nervous system (CNS).

β -Endorphin consists of 31 amino acid residues with a sequence identical to amino acid residues 61–91 in the 91 amino acid peptide of ovine β -lipotropin^{4,5}. The first five amino acids of β -endorphin (residues 61–65 in β -lipotropin) are identical with methionine-enkephalin (Met⁵-enkephalin)¹ and were found to possess opiate activity in receptor binding assays and in electrically stimulated preparations of mouse vas deferens and guinea pig ileum. Subsequent studies have shown that β -endorphin also possesses opiate activity in receptor binding assays and in guinea pig ileum^{6,7}. More recently, it has been found that β -endorphin, in contrast to Met⁵-enkephalin, has potent analgesic properties when administered directly into the brain and assessed in the tail-flick, hotplate, and writhing tests in mice and in the wet shake test in rats⁸. In these tests, β -endorphin was found to be 18–33 times as potent as morphine on a molar basis and its actions were inhibited by the opiate antagonist naloxone. Although also antagonised by naloxone, Met⁵-enkephalin is an extremely weak and short-acting analgesic peptide^{8,9}. In addition, β -endorphin was shown to be 3–4 times more potent than morphine when injected intravenously¹⁰.

The purpose of this study was to compare the abilities of morphine, β -endorphin and Met⁵-enkephalin to inhibit the

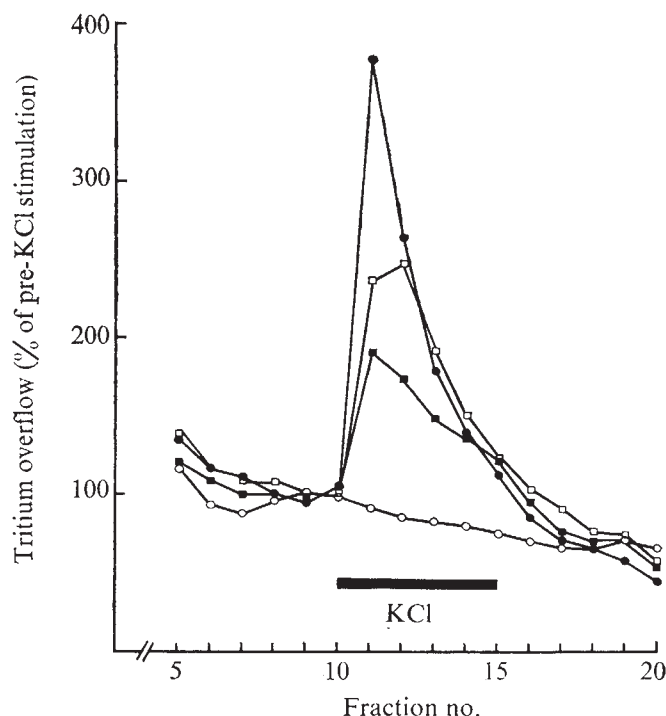


Fig. 1 Concentration-dependent inhibition of potassium-stimulated tritium overflow by β -endorphin. Concentrations of β -endorphin are indicated as follows: 0 μ M ($\bullet$ — $\bullet$); 0.6 μ M ($\square$ — $\square$); 1.2 μ M ($\blacksquare$ — $\blacksquare$); and 3.0 μ M ($\circ$ — $\circ$). Male Sprague-Dawley rats (200–350 g) were administered the monoamine oxidase inhibitor nialamide (100 mg kg⁻¹ intraperitoneally) 1 h before they were killed. Striatal tissue was dissected from a coronal slice of rat brain (1.5 mm thick) and incubated at 37 °C in oxygenated (95% O₂, 5% CO₂) Krebs-Ringer bicarbonate (KRB) buffer (pH 7.4) containing ascorbic acid and 10⁻⁷ M ³H-dopamine, for 20 min. The slices were washed with warm buffer and placed into 13-mm Swinnex Millipore filter holders (1 slice/holder) for superfusion by a 4-channel peristaltic pump. The perfusion rate was 0.5 ml min⁻¹ and 2-ml fractions were collected directly into scintillation vials for counting the tritium. Slices were superfused for 20 min and then superfused with β -endorphin for 20 min before switching to KRB containing 53 mM KCl + β -endorphin (indicated by the horizontal bar), the extra KCl having replaced some of the NaCl in the regular KRB. During the collection of fraction 15, the perfusing solution was switched back to regular KRB. Tritium overflow is expressed as a percentage of the mean of 4 experiments. Preliminary studies with alumina absorption chromatography indicated that approximately 82% of the tritium in regular KRB perfusates was dopamine and this value increased to about 93% in 53 mM KCl KRB perfusates.

Table 1 Opiate inhibition of striatal tritium overflow and reversal by naloxone

Drug	Concentration	Net overflow (%)
Morphine	0	17.69 $\pm$ 2.69 (13)
Morphine	1.0 $\times$ 10 ⁻⁶ M	16.14 $\pm$ 3.42 (8)
Morphine	3.0 $\times$ 10 ⁻⁶ M	5.04 $\pm$ 1.17* (10)
Morphine	1.0 $\times$ 10 ⁻⁵ M	0.41 $\pm$ 0.25* (5)
Morphine + Naloxone	3.0 $\times$ 10 ⁻⁶ M _a	16.85 $\pm$ 3.02 (8)
β -Endorphin	0	20.04 $\pm$ 2.99 (4)
β -Endorphin	0.6 $\times$ 10 ⁻⁶ M	16.76 $\pm$ 3.62 (4)
β -Endorphin	1.2 $\times$ 10 ⁻⁶ M	8.06 $\pm$ 1.60* (4)
β -Endorphin	3.0 $\times$ 10 ⁻⁶ M	0.12 $\pm$ 0.12* (4)
β -Endorphin + Naloxone	3.0 $\times$ 10 ⁻⁶ M _a	10.70 $\pm$ 2.72† (4)
Enkephalin	0	18.09 $\pm$ 2.44 (7)
Enkephalin	1.0 $\times$ 10 ⁻⁵ M	20.99 $\pm$ 4.70 (8)
Enkephalin	1.0 $\times$ 10 ⁻⁴ M	17.23 $\pm$ 4.11 (5)

*Significantly different from control ($P < 0.02$).

†Significantly different from 3 $\times$ 10⁻⁶ M β -endorphin alone ($P \leq 0.01$).

Rat brain striatal slices were used as described in the legend to Fig. 1. Net overflow was calculated from tritium in fractions 11–15 corrected for the estimated spontaneous tritium overflow and is expressed as a percentage (mean $\pm$ s.e.) of the total tritium in the striatal slice at the beginning of the collection of fraction 11. Numbers in parentheses indicate the number of experiments.

potassium-stimulated overflow of tritium from superfused rat brain striatal slices preloaded with ³H-dopamine. Recent studies from our laboratory have shown that potassium-induced ³H-dopamine release from superfused striatal tissue from several strains of mouse which exhibit running activity in response to morphine can be inhibited by 10⁻⁵ M morphine^{11,12}. This inhibition of release seems to be a specific opiate effect because it is antagonised by naloxone, and striatal tissue from mice made tolerant to morphine by the implantation of a morphine pellet for 3 d exhibits tolerance to the inhibitory effect of morphine on dopamine release *in vitro*¹³.

Figure 1 shows the concentration-dependent inhibition of the potassium-stimulated overflow of tritium by β -endorphin from superfused rat striatal slices. Virtually complete inhibition was obtained with 3.0 μ M β -endorphin. In Table 1, the effects of morphine, β -endorphin and Met⁵-enkephalin on the net tritium overflow induced by the depolarising concentration of potassium chloride are compared. β -Endorphin was about twice as potent as morphine, the IC₅₀ for β -endorphin being 1.0 μ M and the IC₅₀ for morphine being 2.2 μ M. Met⁵-enkephalin, on the other hand,

did not produce a significant blockade of the inhibition by morphine and β -endorphin, the blockade being more complete in the presence of morphine than in the presence of β -endorphin.

In the electrically-stimulated guinea pig ileum, Met⁵-enkephalin is approximately equipotent with normorphine¹ or morphine¹³, whereas in the mouse vas deferens it is about 20 times as potent as normorphine¹ or morphine¹³. β -endorphin and Met⁵-enkephalin are approximately equipotent in the guinea pig ileum preparation⁶. In opiate receptor binding assays with guinea pig brain membranes, Cox *et al.*⁶ have shown that synthetic β -endorphin is 2–3 times as potent and Met⁵-enkephalin one-fifth as potent as normorphine. Thus, the order of potency of β -endorphin, morphine and Met⁵-enkephalin in the inhibition of striatal dopamine release seems to follow more closely the order in the binding and analgesia assays than in the assays utilising smooth muscle preparations.

In a very recent paper, Taube *et al.*¹⁴ have described inhibition of noradrenaline release by enkephalin in rat cortex slices. However, to our knowledge, this is the first demonstration of an inhibition of dopamine release from CNS tissue by an endogenous opiate-like peptide. The actions of β -endorphin in the central nervous system are probably not limited to the inhibition of dopamine release because it is known that opiate drugs also inhibit the release of acetylcholine^{15–18} and noradrenaline¹⁹ from CNS tissue. At present, however, dopamine is the only neurotransmitter for which tolerance to the effect of morphine *in vitro* has been demonstrated with CNS tissue¹¹ and for which an inhibition of release by morphine has been correlated, at least qualitatively, with a behavioural effect of morphine, by utilising mouse strain differences^{11,12}.

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Immunochemical evidence of cholecystokinin-like peptides in brain

A NUMBER of peptides including substance P^{1,2}, somatostatin³ and vasoactive intestinal peptide⁴, have been found both in nerve cells of the brain and gut and in gastrointestinal endocrine-like cells. The biological significance of the occurrence of the same peptides in endocrine and nerve cells is not yet clear, although Pearse has suggested that these cell types might share a common embryological (neuroectodermal) origin⁵. An antibody to the antral hormone gastrin has recently been shown to cross react with material in extracts of the central nervous system (particularly cerebral hemispheres) of a number of vertebrate species including dog and man⁶. It is known that in antral mucosa, and in blood, gastrin occurs in several different forms of which the most important are peptides of 17 (G17) and 34 (G34) amino acid residues⁷. The C-terminal heptadecapeptide of G34 is identical with G17, and G34 may well be a biosynthetic precursor of G17 (ref. 7). The C-terminal pentapeptide of the two gastrins is also identical with that of the intestinal hormone cholecystokinin (CCK)⁸, and antibodies specific for the C terminus of gastrin frequently cross react with CCK⁹. The present study was undertaken to clarify the relationships between the gastrin-like activity in brain and the characteristic forms of gastrin and CCK. We found the distribution of the brain components on Sephadex G-25 to differ from those of previously characterised forms of gastrin and CCK. The pattern of reactivity with different antisera suggests, however, that the brain factors resembled CCK-like peptides more closely than gastrin-like peptides.

Extracts were prepared from the entire brain of rats, and from the cerebral cortex of hog and dog. Small pieces of tissue were boiled in water (0.5 g ml⁻¹) for 3 min to inactivate enzymes, homogenised, centrifuged (2,000g, 20 min) and the supernatant fractionated on calibrated columns of Sephadex G-25 or G-50. Immunoreactivity in the column eluates was estimated by radioimmunoassay using a C-terminal-specific antiserum (1296) (ref. 10).

Three peaks of immunoreactive material were found in column eluates when extracts of hog brain were fractionated on Sephadex G-25 (Fig. 1); similar results were obtained with extracts of rat and dog brain. The first peak (BP I) emerged in the void volume and accounted for 10–15% of the total immunoreactivity. Standard samples of G34 and CCK also emerged in the void volume on Sephadex G-25. But BP I differed from these standards in that it also emerged in the void volume after refractionation on Sephadex G-50, whereas G34 and CCK emerged later on Sephadex G-50 (approximately 35% and 50% respectively of total elution volume). In extracts of jejunum, and in plasma, there is material with gastrin-like immunoreactivity which emerges in the void volume on Sephadex G-50, and this component has been named 'big, big gastrin' (BBG) (ref. 11). Trypsin converts BBG to a peptide resembling G17 (ref. 11). In contrast, preliminary experiments indicated that when BP I was digested with trypsin (100 µg ml⁻¹, 10 min) there was conversion to material emerging later than G17 on Sephadex G-25, at 60–90% of elution volume. So far only relatively small amounts of this product have been available and the identity of the material is not yet clear. Nevertheless the evidence indicates that BP I does not correspond to any of the previously characterised forms of gastrin or CCK.

The main peak of immunoreactivity in the brain extracts (BP II) eluted from Sephadex G-25 in a similar position to the C-terminal octapeptide of CCK (CCK 8); this peak accounts for over 70% of the total immunoreactivity (Fig. 1). A third peak of activity (BP III) representing 10–15%

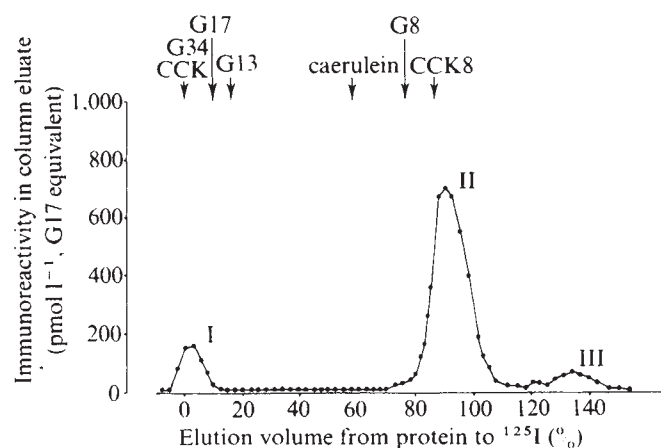


Fig. 1 Fractionation of an extract of hog brain on Sephadex G25 superfine (1 × 100 cm). Fractions of 1.0 ml collected every 10 min. Column eluted with 0.02 M sodium barbital pH 8.4, containing 0.02% sodium azide, at 4 °C. The column was previously calibrated with 20% pure porcine CCK, pure natural human G34-I and G17-I, caerulein, synthetic peptides corresponding to the C-terminal tridecapeptide (G13) and octapeptide of G17-I (G8), and CCK 8. The elution volumes of the standards are indicated by arrows. In all column runs the void volume was marked by protein estimated by absorption at 280 nm, and the salt region was marked by Na-¹²⁵I. Elution volume is expressed as percentage from void (0%) to ¹²⁵I (100%). Concentration of immunoreactive material was estimated with antiserum 1296 and expressed relative to standard G17. Peaks of immunoreactivity are identified by Roman numerals.

of the total immunoreactivity emerged considerably later than Na¹²⁵I (Fig. 1). Synthetic peptides corresponding to the C-terminal hexapeptide and tetrapeptide of G17 were found to emerge in the same region as BP III, but the standards were poorly resolved and it has not been possible to relate precisely their mobility to that of BP III.

The immunochemical properties of the components in brain were studied by separately pooling fractions corresponding to the three peaks and estimating concentrations of immunoreactive material by radioimmunoassay using six different antisera. The specificities of the antisera had been established by studies of the potency of standard molecular forms and synthetic fragments of gastrin and CCK in inhibiting binding of ¹²⁵I-labelled G17 to antiserum. Only antisera specific for the C terminus of gastrin and CCK cross reacted with the brain components. Antiserum L6, which has specificity for intact G17 (ref. 12), and antiserum 1295, which is specific for the N terminus of G17 (ref. 10), failed to cross react with extracts of the brain of rat, dog or hog, either before or after fractionation by gel filtration (Table 1). Neither 1295 nor L6 cross reacted with rat antral

gastrin so their inability to detect immunoreactive material in rat brain is not surprising. Both antisera did, however, cross react with dog and hog G17 and so the failure to detect activity in brain extracts from these species indicates that G17 could be present only in minute quantities, if at all, in the extracts. These results are therefore consistent with the failure to find a peak of immunoreactive material eluting in the same region as standard G17 when brain extracts were fractionated on Sephadex G25 (Fig. 1).

When G17 was used as a standard in the radioimmunoassays there were up to 100-fold differences in concentrations of brain components estimated with different antisera (Table 1). For each of the components the highest concentrations were recorded with antiserum C. This antiserum was raised against CCK 8 and shows twofold higher immunoreactivity with CCK 8 than with G17. On the other hand the lowest values were recorded with antiserum L2, which was raised against porcine G17 and shows 50–100 times higher immunoreactivity with G17 than with CCK 8. Two other antisera (1296, 2716) gave intermediate estimates of the concentration of brain components; both of these antisera were raised against human G17 and show 5–15 times higher immunoreactivity with G17 than with CCK 8.

Because the pattern of immunoreactivity of the brain extracts seemed to follow the degree of specificity of the antisera for CCK, the results have also been expressed relative to a CCK standard. Pure CCK was not available and so CCK 8, which contains the minimal active fragment of CCK for biological activity, was used instead. Table 1 shows that there were only relatively minor differences in estimates of immunoreactive concentration of brain components made with different antisera when based on the CCK standard. The differences between antisera were more pronounced for BP I (two- to six-fold) than for BP II and III (one- to three-fold). Taken together the results indicate that the brain components have a pattern of immunoreactivity resembling CCK-like peptides more closely than gastrin-like peptides.

An attempt to isolate these brain peptides has been initiated in this laboratory; until this is achieved absolute estimates of their concentration in brain cannot be made. Expressed in terms of CCK 8 equivalent, however, the main component (BP II) was present in amounts of 50–150 pmol g⁻¹ in the brain of the three species (Table 2). It is unlikely that concentrations such as these could be produced other than by synthesis within the CNS. Many peptide hormones are synthesised in the form of large precursor molecules which are converted to smaller biologically active forms by proteolytic enzymes. The present results could therefore be accounted for if the three components were the product of a single biosynthetic pathway and interconversion took place by proteolysis. It is clear that the cellular origins of these components must now be studied. In addition, attention must be given to the biological activity of

Table 1 Concentration of three fractions of hog brain estimated by radioimmunoassay with six antisera

Fraction	Standard	C	2716	Antiserum		1295	L6
				1296	L2		
Concentration (pmol l ⁻¹)							
Hog BP I	G17	156	34	34	8	<2	<2
	CCK 8	78	100	212	472	—	—
Hog BP II	G17	2,341	177	119	23	4	2
	CCK 8	1,170	520	735	1,355	—	—
Hog BP III	G17	130	14	13	1	<2	<2
	CCK 8	65	41	81	58	—	—

Each figure is the mean of assays on three separate samples of brain extracts. Samples were pooled from eluates of Sephadex G25 columns. Results are expressed relative to standard pure natural human G17-I and synthetic C-terminal octapeptide of CCK (CCK 8). ¹²⁵I-labelled human G17 was used as label in all radioimmunoassays. With all four C-terminal specific antisera (C, 2716, 1296, L2) dilution curves for the three brain components were parallel to those for G17 and CCK 8. Antisera 1295 and L6 do not cross react significantly with CCK 8 and so for these antisera results have been expressed relative to standard G17 only.

Table 2 Concentration of immunoreactive components in extracts of the cortex (hog and dog) or whole brain (rat)

Species	n	Concentration of components in brain (pmol g ⁻¹ : CCK 8 equivalent)		
		BP I	BP II	BP III
Hog	5	16.9±3.1	95.0±33.0	11.2±1.1
Dog	3	14.4±3.7	58.1±20.6	18.1±7.5
Rat	5	30.6±6.9	149.0±20.6	27.5±6.2

Radioimmunoassays were performed using antiserum 1296 and results expressed relative to standard CCK 8. Concentrations were estimated by integration of peaks in Sephadex G25 eluates and calculated on the basis of wet weight of tissue from which the extract was prepared.

the components and, by analogy with other CNS peptides (substance P, somatostatin), a possible role as neurotransmitters or releasing factors deserves consideration.

Technical assistance was provided by Mrs Elaine Clark, Miss Carol Higgins and G. Laing. Antisera C and 2716 were gifts from Drs S. Bloom and J. Rehfeld, respectively. Standard peptides were gifts from Professor R. A. Gregory and Dr H. J. Tracy (G34 and G17), Professor G. W. Kenner (C-terminal octapeptide, hexapeptide and tetrapeptides of G17), Dr V. Mutt (20% porcine CCK), Squibb Institute of Medical Research (CCK 8), and Farmitalia Ltd (caerulein). This work was supported by a grant from the MRC.

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Visual contrast sensitivity of a 6-month-old infant measured by the evoked potential

THE visual contrast sensitivity of the human infant has previously been measured using behavioural methods, in which psychophysical thresholds have been inferred from visual preferences¹. In this paper we report a preliminary investigation of contrast sensitivity, as measured by the amplitude of the evoked cortical response, in a 6-month-old infant. These results are compared with behavioural measurements on the same infant, and with adult data obtained psychophysically and from evoked cortical responses.

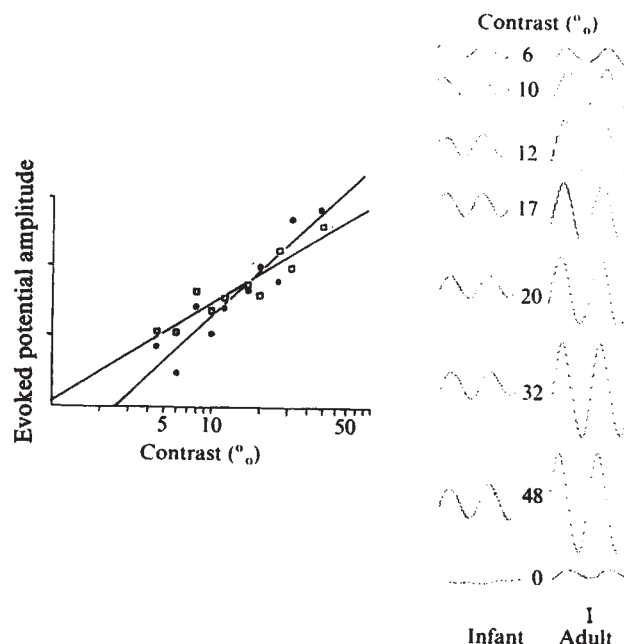
Campbell and Maffei² measured the visual evoked cortical response to a phase reversing sinusoidal grating and found that a linear relationship existed between the logarithm of contrast and the amplitude of the evoked potential. By extrapolating a regression line through such a plot to zero voltage, the psychophysical threshold could be predicted³. This relationship found in adult evoked potential measurements has been used in the present study to indicate thresholds for contrast in a situation where direct psycho-

physical methods are not possible, that is, in human infants.

The stimuli were vertical gratings of sinusoidal luminance profile, whose phase was reversed at 10 Hz. They were generated on an oscilloscope screen by established methods^{4,5}, with a mean luminance of 300 cd m⁻², and subtending an angle of 20° at 57 cm. To ensure that evoked potentials for different contrast levels were comparable in spite of possible artefactual changes over time, the various contrasts for a given spatial frequency were presented repeatedly in short blocks randomly interleaved within a particular run, which lasted about 10 min. Each block lasted 5.1 s. Control blocks, in which the contrast was set to zero, were included in each run. The infant was held sitting 57 cm from the display screen (110 cm for runs with spatial frequency higher than 5 cycles per degree). Between the infant and the display there was a 70% reflecting mirror arranged to superimpose the image of an active face on the stimuli, which served to maintain the infant's attention.

Evoked potentials were recorded from both the infant and the adult by means of 2-mm Beckmann electrodes. One electrode was placed 1 cm above the ionion and the other 3 cm temporally: the signals from these were differentially amplified 100,000 times, with an earth electrode placed behind the ear. The amplified signal was filtered (by a Kemo VBF/3/J filter) with a passband of ±2 Hz centred on the second harmonic of the frequency of phase alternation (20 Hz). The filtered signal was fed into an analog input channel of a PDP-11/10 computer which maintained separate averages of the signal for each contrast level used, and also controlled the stimulus contrast and phase alternation. These averages were of a 100-ms sweep and therefore contained two cycles of the second harmonic signal. The first 100 ms of each 5.1-s block were not included in the average, to avoid the effect of initial transients due to the contrast change. The averaging could be interrupted when the infant was not attending to the screen.

Fig. 1 Right, averaged evoked potentials (500 sweeps) for a series of contrast levels, from the infant and adult subjects. Spatial frequency: 0.3 cycles per degree. The bottom traces were obtained with no grating present. Scale: 200 × 10⁻⁹ V per sweep (adult), 50 × 10⁻⁹ V per sweep (infant). Left, plot of amplitude of these potentials against contrast (logarithmic scale). ●, Adult data; □, infant data. Regression lines are shown for each subject. The amplitudes have normalised for this plot.



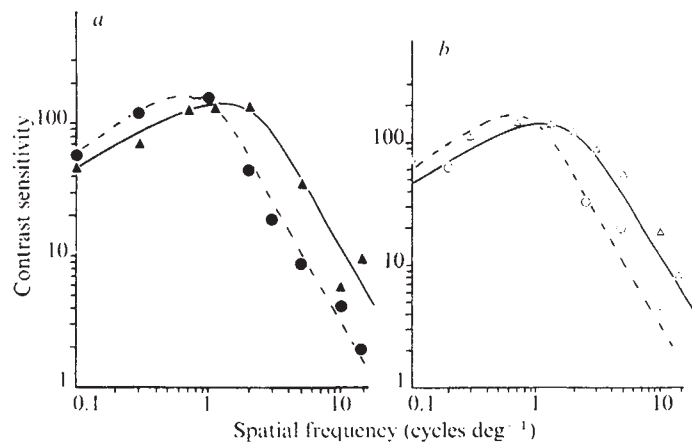


Fig. 2 *a*, Comparison of the contrast sensitivity functions for the adult (Δ) and infant ($\bullet$), as derived from the amplitude of the evoked potential. The curves are fitted by eye. Each point is an average of several runs. *b*, Comparison of the psychophysical contrast sensitivity for the adult (Δ) and the behaviourally determined contrast sensitivity function for the infant ($\circ$). The curves from Fig. 2 *a* are repeated in this figure to aid comparison between psychophysical and evoked potential measures.

In general 500 sweeps were averaged at a given contrast, although for some low contrasts extra blocks were included in the program to obtain a signal of sufficient amplitude. The averaged signals, which because of the narrow pass-band were approximately sinusoidal, were displayed, plotted on an X-Y plotter, and the peak-to-trough amplitude was put out by the computer. Signals have only been included in the results if their peak-to-trough amplitude was at least 20% higher than that measured in the average for the zero contrast control blocks.

The subjects were tested for responses to spatial frequencies ranging from 0.1 to 15 cycles per degree, and contrasts ranging from 4% to 50% for each frequency. Psychophysical thresholds for the adult were measured with the same apparatus, using a yes-no forced choice method with a double random staircase procedure under the computer's control. The trials near to threshold were interleaved with high contrast displays in a similar sequence to that used for the evoked potential measurements and the screen was viewed through the 70% mirror. Behavioural data on the infant were gathered using the method of fixation preference assessed by two-alternative forced choice by a 'blind' observer⁶. Stimuli were presented on two oscilloscopes at a distance of 40 cm from the infant, subtending 15° each and separated by 9°. One screen displayed a horizontal test grating and the other a blank field matched in mean luminance. The behavioural threshold was defined as the contrast level at which the observer could make 70% correct judgements of the side to which the stimulus was presented. This was obtained by a modified staircase procedure.

Refraction of the infant subject showed that he was about 1 D hypermetropic with 0.75 D of astigmatism in each eye. He should therefore have had no difficulty accommodating to the stimulus distance. The adult subject was emmetropic.

Figure 1 shows a series of averaged signals for a single spatial frequency (0.3 cycles per degree) and increasing contrast for both the infant and adult subjects. The peak-to-trough amplitudes of the signals of each series were plotted against log contrast and the linear regression line extrapolated to give the contrast for zero amplitude. These extrapolated contrasts are plotted in Fig. 2*a* as a function

of spatial frequency, for both the infant and the adult subjects. Curves have been fitted to these by eye. Plotted in Fig. 2*b* are the psychophysically or behaviourally obtained contrast sensitivities for each subject, with the curves of the evoked potential results from Fig. 2*a*.

For the adult, the extrapolated evoked potential "threshold" estimates lie close to, but in most cases slightly below, the psychophysical thresholds. This is compatible with previous findings^{2,3}. The behavioural measure on the infant gives a lower limit on the estimate of sensitivity. This in fact coincided rather closely with that derived from the evoked potential measurements (Fig. 2*b*).

In the case of the low spatial-frequency stimuli, it is not possible to determine from our data whether the evoked potentials are generated by the spatial pattern, or by a frequency-doubled response to flicker⁷. When modulation is both spatial and temporal, however, psychophysical thresholds can also be distinguished by the detection of either pattern or flicker^{8,9}. The uncertainty about the origin of the evoked potential does not therefore exclude a comparison with the behavioural data, but it does indicate a need for caution in extending the results to different temporal conditions, such as a static pattern.

The use of evoked potentials allows us to compare the same measure of sensitivity, employed on both infant and adult. Figure 2*a* shows that for low spatial frequencies the contrast sensitivity derived in this way is very similar for both subjects. At spatial frequencies of 2 cycles per degree and above, however, the adult is shown to be markedly more sensitive. In making these comparisons, it should be borne in mind that the infant and adult visual systems may show differences in temporal as well as spatial response.

We therefore conclude: (1) that the neural mechanisms determining contrast sensitivity at low to medium spatial frequencies have matured by 6 months of age to close to the adult level (this is consistent with the finding¹⁰⁻¹² that the optimum check size for checkerboard-evoked potentials is similar in the 6-month-old and the adult). However, the neural mechanisms determining high spatial-frequency sensitivity are not fully mature at this age. (2) Measurements of contrast sensitivity by means of the visual evoked potential are possible in infants and give data consistent with behavioural methods. It may prove a valuable clinical tool for the early diagnosis of visual problems.

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The 'cerebellum' and the control of eye movements in cephalopods

THE cybernetic principles involved in the control of locomotion in vertebrates include feed-forward and feedback between the optic and vestibular systems, through a series of control centres, including the cerebellum. Cephalopod molluscs also show swift and well controlled locomotion and have eyes and static organs, but there is little detailed evidence as to how the information from these controls movement. The basal lobes of the supraoesophageal part of the brain are known to be higher motor centres, but this is, at best, a vague term. The new studies of these lobes and of the statocyst by my colleagues and I that are reported here show more clearly the part they play in the control of behaviour. Their organisation proves to be basically similar to that of the comparable systems of vertebrates.

To the many parallels between the organisation of cephalopods and vertebrates described by Packard¹ we can therefore now add that the control of movement of the eyes and body involves a system similar to the cerebellum. The resemblances and the differences between the two systems may help towards understanding of the principles of movement control. In both there is a direct feed-forward from angular acceleration receptors to the oculomotor centre and a visual feedback system involving numerous fine parallel fibres (Fig. 1). From the verte-

Fig. 1 *a*, Diagram of the oculomotor control system in the squid arranged to show its correspondence to that of a mammal. *b*, The oculomotor control system in a mammal (copied, with permission, from Ito²). AOT, Accessory optic tract; CF, climbing fibre; CTTm, central tegmental tract; GR, granule cell; IO, inferior olive; MF, mossy fibre; PCJ, Purkinje cell; WB, unidentified brainstem nucleus; VN, vestibular nucleus.

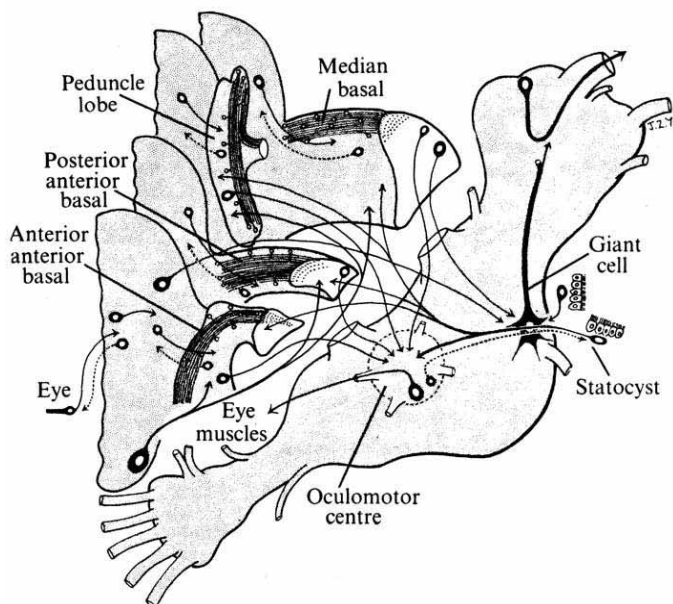
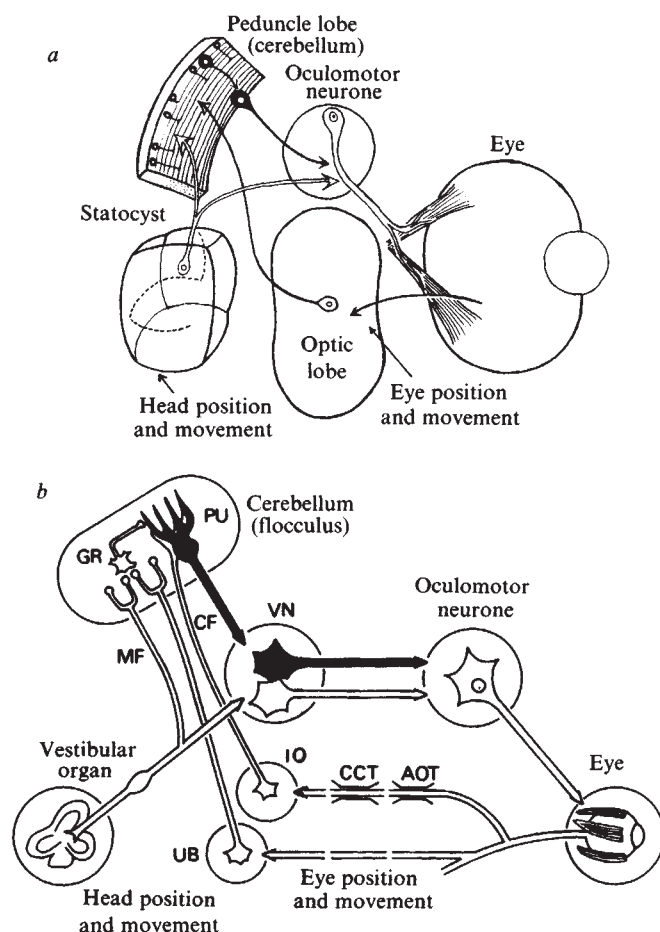


Fig. 2 Diagram of the eye-muscle control centre and basal lobe system of the squid, *Loligo*. Return centrifugal pathways are shown dotted. Note that they reach to the retinal and statocyst receptors.

brate vestibular organs, the pathway is through the vestibular nucleus²; in cephalopods fibres from the statocyst itself end in the oculomotor centre in the lateral pedal lobe (Fig. 2). It is these fibres that must be acting in the fast phase of nystagmus in *Sepia*³. In both groups the vestibular information reaches the eye muscles also through a second route in which it is combined with visual information. In vertebrates this route is through the flocculus of the cerebellum, whose Purkinje axons have been shown to exert an inhibitory influence on the neurones of the vestibular nucleus in rabbits⁴. In cephalopods the corresponding pathway is from the statocyst to each member of a set of basal lobes and from there direct to the oculomotor neurones. The visual input to the vertebrate cerebellum comes through the accessory optic tract and the inferior olive and also by mossy fibres by way of another nucleus (Fig. 1)⁵. Stimulation of the optic disk inhibits the vestibular neurones with a delay of 12 ms and of the inferior olive after 3 ms². In cephalopods the visual pathway is again through the basal lobes and there is good evidence of optokinetic nystagmus^{3,6,7}. Electrical stimulation of the basal lobes produces movements of the eyes, as well as of the head, arms and fins⁸.

Both vertebrates and cephalopods thus use a direct feed-forward control from the statocyst and a feedback from the eyes through cerebellar-type systems. The experiments of Messenger and Collewyn show clearly that the two influences together are necessary for steady visual control. They constitute a classical set of evidence for the effectiveness of such a double system.

The functional parallels between vertebrates and cephalopods make it especially interesting that the cephalopod basal lobes resemble the cerebellum in structure. There has been evidence for some time that the parallel fibres in the peduncle lobe, situated on the optic tract of cephalopods (shown in Fig. 3) resemble the granule cells of the cerebellum⁹⁻¹¹. It has now become clear that this lobe is part of a much more extensive system including the basal lobes of the brain, serving to regulate movement of the eyes and of the whole animal. The arrangement is similar in octopuses and squids but the plan has become much clearer by a recent study of squids, where some of the fibres are larger¹².

The system consists of four lobes on each side of the animal, each with a different orientation (Figs 2 and 4). The outstanding feature of each lobe is a set of parallel fibres. The region known as the anterior basal lobe contains two of these sets of

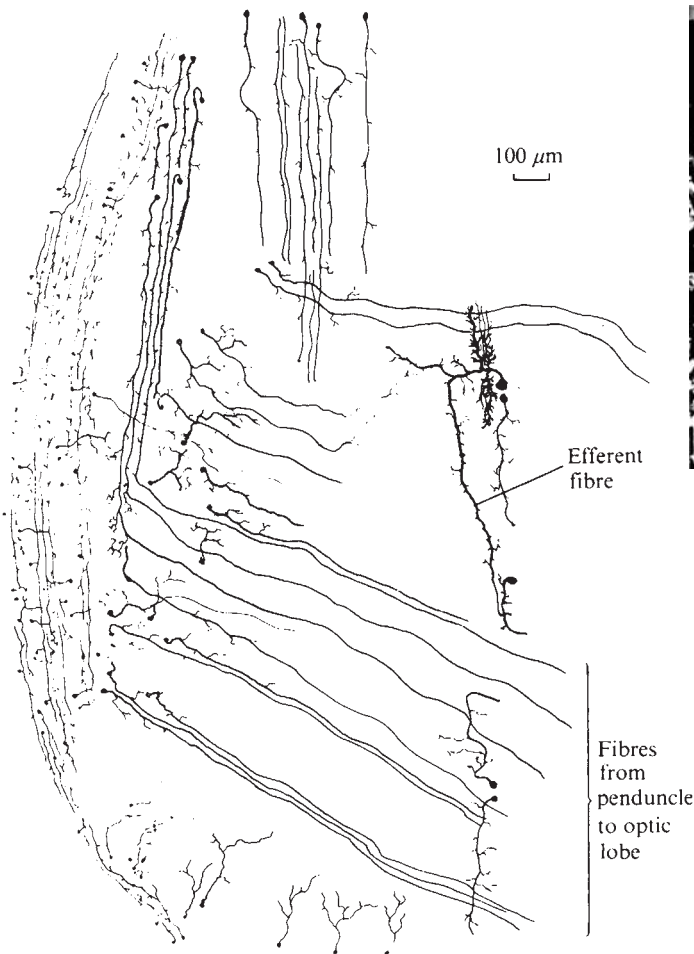


Fig. 3 Parallel fibres of the peduncle lobe of the squid (*Loligo*). Drawing from a Golgi preparation¹⁰. The optic lobe lies to the right and fibres are shown running to it. The large cell carries an output fibre with axon proceeding to the oculomotor or other motor centre. The input fibres from the optic lobe and statocyst are not shown.

fibres lying in the transverse plane. One set, the posterior anterior basal, runs transversely. The other, the anterior anterior basal, runs across the midline and then down vertically at the sides. A third set is in the median basal lobe, which although it is a more complicated region has similar parallel fibres running transversely, approximately in the horizontal plane. The fourth set of parallel fibres is in the peduncle lobe, running dorsoventrally in the sagittal plane in squids but antero-posteriorly in octopods. The four lobes together thus make a sort of 'cerebellum' with motor control functions.

These lobes all receive fibres from the optic lobes and from the statocysts (Fig. 2). They send fibres to the motor centres for the movements of the eyes and body, and others back to the optic lobes. They thus have essentially the same functional relations as the cerebellum, lying between vestibular and optic centres and serving for integrated visuostatic control of movement. It is therefore very striking to find that there are also similarities of internal organisation¹¹. The inputs of each lobe of the system in cephalopods may be listed thus. (1) Dorsal optic fibres apparently preserving the somatotopic plan of the optic lobe; it is suggested these may serve for tracking. (2) A ventral optic input of a few very large fibres from a region at the base of the optic lobe, perhaps involved with initiating attack. (3) Fibres from the statocyst. (4) Fibres from the giant cell lobe, perhaps related to escape movements. (5) Numerous fibres from the corresponding opposite lobe. (6) Numerous fibres from each of the other basal lobes.

The output is not quite the same for all the lobes but each sends to the following destinations. (1) To the oculomotor centre controlling the four eye-muscle nerves. (2) To the regions con-

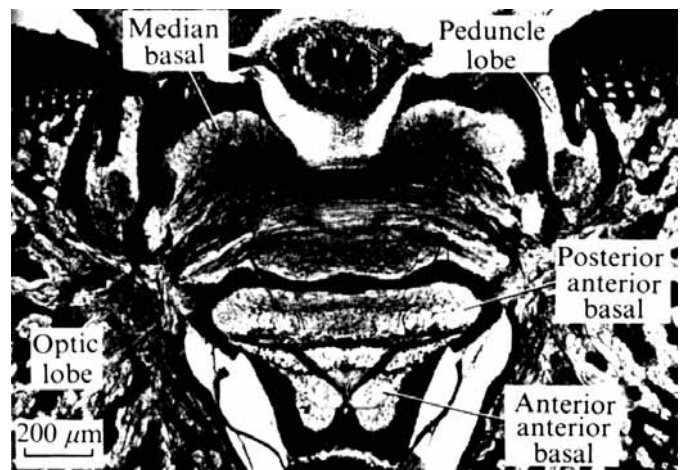


Fig. 4 Horizontal section of the supraoesophageal lobes of a small *Loligo*. Cajal stain, showing the positions of the four basal lobes on each side.

trolling the funnel and fins (for steering). (4) To the giant cells that initiate the jet. (5) A reverse projection to the optic lobe, allowing for 'efference copy' ('corollary discharge')¹³⁻¹⁵.

The details of the organisation within the peduncle lobes are beginning to be known^{9,10,16}. Some of the parallel fibres are the T-shaped processes of small "granule" cells and may run for the width of the lobe (1–2 mm) (Figs 5 and 6). They are about

Fig. 5 Golgi stained preparation of peduncle lobes of the small squid *Alloteuthis*, showing the fine parallel fibres and others crossing them.





Fig. 6 Golgi preparation of *Alloteuthis*. Retouched photograph showing especially the collateral dendrites of the fibres that cross the parallel-fibre bundles.

0.3 μm in diameter and carry a series of collaterals, some making synapse with other parallel fibres. The afferents from the optic lobes branch among the bundles. Other cells have fibres that turn down across the parallel fibres to the basal region, from which the output fibres of the lobe arise (Fig. 6). These may thus serve to receive signals from the parallel fibres and pass them to the output cells, but the details of the connectivity are not yet clear. The parallel fibres in the anterior basal lobes are so similar to those in the peduncle lobes in transmission electron micrographs that it is difficult to distinguish between them¹⁶. All the sets of parallel fibres have a characteristic fluorescence, possibly due to 5-hydroxytryptamine, which is not found elsewhere in the brain¹⁷.

Evidence from electrical stimulation and surgical removal confirms that these lobes are concerned with the control of movement of the eyes and body^{9,18,19}. After unilateral lesions in the anterior basal lobe, persistent circling with the lesioned side at the centre has been seen in *Sepia*¹⁸ and *Octopus* (personal observations). After the bilateral removal of the peduncle lobes in *Octopus* there are various motor defects and movements are jerky and less precise^{9,19}.

The pattern of control of movement of the eyes and of the whole animal thus shows remarkable similarities between cephalopods and vertebrates, in spite of the fact that the gross anatomical entities are totally different. The need to monitor the animal's own movements is presumably met by the signals from the cristae of the statocyst, which run in distinct planes, providing information about angular accelerations^{20,21}. The presence of the extensive reverse pathways from the centres in the basal lobes to the optic lobes meets the need to compare performance with internally generated goal specifications. It has been common since the work of von Holst to regard this as a (relatively simple) process of subtraction. As pointed out by MacKay¹³, however, the process may be much more complex than this and it is interesting that the information is sent to the optic lobes, which probably act as what he would call a sensory evaluator.

The presence of systems of parallel fibres is especially intriguing and it is tempting to relate it to the hypothesis of Braitenberg²² that such systems provide for the timing of ballistically controlled movements, which are of course a marked feature of cephalopod behaviour. Whatever may be

their function the fine parallel fibres seem to be an essential feature of such systems.

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Effect of riboflavin on red-cell metabolism of vitamin B₆

THE red cell is an active site for the conversion of pyridoxine to physiological forms of vitamin B₆. Pyridoxine phosphate is first formed by a kinase and then converted to pyridoxal phosphate by an oxidase, followed by dephosphorylation to pyridoxal, the form which is released into plasma¹. In a large proportion of the patients with homozygous or heterozygous β -thalassaemia whom we have studied, there was a much reduced *in vitro* rate of red-cell conversion of pyridoxine, a familial characteristic inherited separately from thalassaemia, and also found in some haematologically normal subjects²⁻⁴. This rate of conversion, whether reduced or normal, is increased by incubation of blood with riboflavin⁴, possibly through synthesis in the red cell of flavin mononucleotide (FMN) from riboflavin⁵, which then stimulates the pyridoxine phosphate oxidase⁶, a flavoprotein⁷. In order to study the *in vivo* effect on red-cell metabolism of B₆, riboflavin was administered to one of the haematologically normal subjects with a greatly reduced red-cell conversion rate of pyridoxine. The rate increased to normal within 3 week. Here we report the details of this study.

The *in vitro* red-cell conversion of pyridoxine was studied by incubating 500 ng pyridoxine (as pyridoxine-HCl; Sigma) per ml of blood at 37 °C for periods up to 2 h as described previously¹. The *L. casei* microbiological assay was used to measure pyridoxal and pyridoxal phosphate^{1,8}. Pyridoxal phosphate cannot be measured as such, for the *L. casei* measures only pyridoxal directly. Therefore, blood was first acid-hydrolysed in order to free pyridoxal phosphate from protein and to convert it to pyridoxal. The sum of pyridoxal phosphate plus pyridoxal was therefore measured in a hydrolysate and represents the total conversion to active forms. This was expressed as a percentage of the added pyridoxine.

The subject studied was a healthy woman aged 65 yr with a haemoglobin concentration of 1.4 g dl^{-1} and with no evidence of thalassaemia. Her red-cell conversion rate was the slowest of five "slow converters" found among the control subjects. On two occasions with an interval of a year the amount converted was 1.9% and 2.3% per g $\text{Hb} \times 10^{-2}$ at 1 h which was similar to the lowest values found associated with thalassaemia^{3,4}. Her son and daughter had values intermediate between those of their mother and their father whose conversion of pyridoxine was normal. In *in vitro* studies riboflavin or FMN was incubated with blood of the subject and her son, and both compounds significantly stimulated the conversion of pyridoxine.

The *in vitro* red-cell conversion of pyridoxine in the subject was measured immediately before she started a course of riboflavin (Fig. 1). She took 10-mg tablets twice a day for 8 weeks. The conversion of pyridoxine to active forms was measured 1, 3 and 8 weeks after starting this treatment. She then stopped taking riboflavin and the conversion was measured at intervals over 17 weeks. The amount of pyridoxine converted to active B_6 forms after incubation for periods up to 2 h is compared for eight of 14 tests done during the 25 weeks (Fig. 1), and these are compared with the mean amount converted in 60 control subjects.

After one week on riboflavin the rate of conversion of pyridoxine had markedly increased. The amount converted by 1 h increased from 33 to 53%, and after 3 weeks had increased further to 87%, which was equivalent to 6.2% per g $\text{Hb} \times 10^{-2}$ and was well within the normal range (5.0 to 8.6 mean 6.6% per g $\text{Hb} \times 10^{-2}$). After 8 weeks on riboflavin the conversion of pyridoxine in this subject was above the mean found in control subjects. After stopping riboflavin, the rate decreased considerably within the first 3 weeks, and continued to decrease steadily. Only by the seventeenth week, however, had the amount of pyridoxine converted at 1 h (2.3% per g $\text{Hb} \times 10^{-2}$) dropped as low as before treatment. It is interesting that the duration of her increased conversion rate corresponds approximately to the normal lifespan of red cells.

The fact that riboflavin administration resulted in a marked increase in the red-cell conversion rate of pyridoxine in this subject strongly suggests that pyridoxine phosphate oxidase is the defective enzyme. A similar though probably more rapid pattern of activation of another red-cell flavin enzyme, glutathione reductase, has been shown after ribo-

flavin administration either when the enzyme activity was originally normal or was reduced⁹. But the prosthetic group for glutathione reductase is flavin adenine dinucleotide (FAD) whereas for pyridoxine phosphate oxidase (an enzyme equally effective in the oxidation of pyridoxamine phosphate¹⁰) it was found to be FMN¹¹. The prompt response of both these enzymes to riboflavin administration is presumably explained by the fact that mature red cells take up riboflavin and synthesise the riboflavin nucleotides, FAD and FMN⁵.

The significance of the ability of the red cells to convert pyridoxine to physiological forms of vitamin B_6 is not yet understood. The considerable uptake of pyridoxine by red cells, far in advance of the rate of conversion^{1,4} suggests that a significant proportion of ingested pyridoxine is metabolised by the red cell (6% of a 40-mg oral dose as in the red cells at 20 min as well as another 3% which has passed into the plasma most probably after conversion in red cells). Vitamin B_6 is known to take part in the transport of amino acids in and out of cells^{12,13} and the red cell plays an important role in the transport of amino acids¹⁴. It is therefore possible that the conversion of pyridoxine (and pyridoxamine)¹ in the red cell could be linked to amino acid transport as has been discussed at length in another paper⁴.

In vitro studies in control subjects with normal conversion rates and in patients with thalassaemia with reduced rates demonstrated an increase in the red-cell conversion rate of pyridoxine after incubation of blood with riboflavin or FMN. Detailed *in vivo* studies with riboflavin are not yet completed in these subjects, but we have shown that in β -thalassaemia the rate increases to normal in both heterozygotes and homozygotes after oral administration of riboflavin, as in the subject reported here. In a previous study we found that the red-cell conversion rate of pyridoxine was usually normal in mild homozygous β -thalassaemia (thalassaemia intermedia) but apparently was reduced in the majority of patients with transfusion-dependent β -thalassaemia¹. It is conceivable that the correction of this defect by administration of riboflavin may have some beneficial effect.

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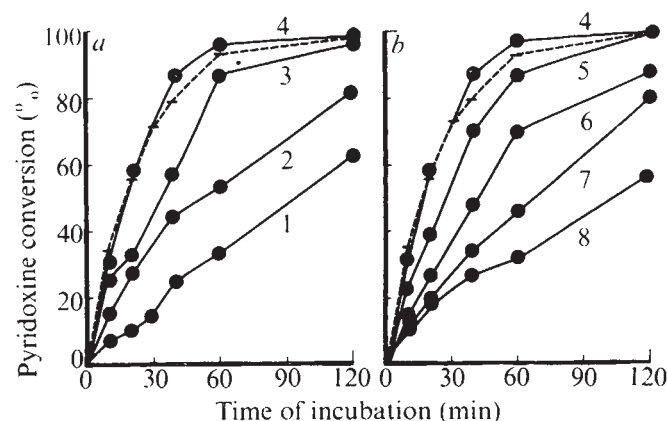


Fig. 1 *In vitro* red-cell conversion of 500 ng pyridoxine per ml blood to *L. casei* active B_6 (pyridoxal phosphate plus pyridoxal), before, during and after oral administration of riboflavin (10 mg twice daily) in a control subject with an initial slow conversion rate of pyridoxine. *a*, During treatment: (1) Pre, (2) 1 week, (3) 3 weeks, (4) 8 weeks. *b*, After treatment: (4) Pre (8 weeks treatment), (5) 1 week, (6) 3 weeks, (7) 9 weeks, (8) 17 weeks. The broken line represents the mean percentage converted by control subjects.

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Polymerisation of membrane tubulin

AMPLE evidence now exists that, in addition to soluble, cytoplasmic tubulin, many tissues contain variable proportions of particulate tubulin as judged from colchicine-binding studies¹⁻⁶. This colchicine-binding activity is not contamination from cytoplasmic microtubules, and is associated with a fraction enriched in plasma membrane markers⁶. The activity exhibits the ligand-binding properties of purified tubulin and reacts with antibodies against soluble brain tubulin. Finally, when the binding activity is solubilised from membranes it exhibits normal binding behaviour toward antimetabolic agents, normal thermal lability, and normal interaction with antibody⁶. What was not clear was whether this tubulin could participate in polymerisation reactions to form microtubules. We report here that solubilised, labelled tubulin copolymerises with cytoplasmic tubulin through repeated cycles of polymerisation and depolymerisation.

Male guinea pigs of strain 13-N (250–300 g) were injected intraperitoneally with 5 mCi ³H-leucine (61 Ci mmol⁻¹) and whole brains were collected 60 h and 8 d thereafter for use in experiment 1 and 2, respectively. The brains were homogenised in medium containing 0.25 M sucrose, 1 mM dithiothreitol and 3 mM Tris-HCl buffer, pH 7.4. Membranes were prepared as previously described⁶ with additional washes to remove possible cytoplasmic contaminants. To check for completeness of removal of such contamination ³H-leucine-labelled cytoplasmic brain tubulin was added to unlabelled brain homogenates from which membranes were subsequently prepared. All soluble tubulin was removed after the third wash of the membranes (Table 1). In agreement with our earlier studies⁶, it seems that tubulin found in the membrane fraction is firmly attached and not an artefact of the isolation procedure.

Fig. 1. Copolymerisation of solubilised membrane-bound tubulin with cytoplasmic rat brain tubulin. The ³H-labelled guinea pig brain membranes (5.2 mg ml⁻¹), isolated as described in Table 1, were suspended in 0.4% of Nonidet P-40 at 4 °C for 30 min and then centrifuged at 100,000g for 20 min at 4 °C. The supernatant, which contained solubilised membrane-bound tubulin, was mixed with an equal volume of 100,000g supernatant of rat brain homogenate and to the total mix an equal volume of 8 M glycerol in polymerisation buffer was then added. The polymerisation and depolymerisation was carried out according to Shelanski *et al.*⁷ and repeated seven times. After each cycle of polymerisation the pellet, which contained microtubules, was suspended in the minimum amount of polymerisation buffer. An aliquot was counted for radioactivity and protein was determined. Experiments 1 (curve *a*) and 2 (*b*) were carried out with guinea pig brain labelled for 60 h and 8 d, respectively.

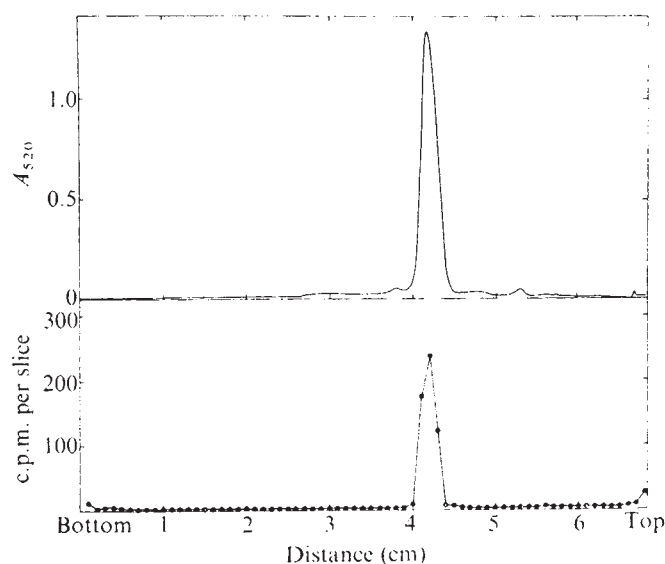
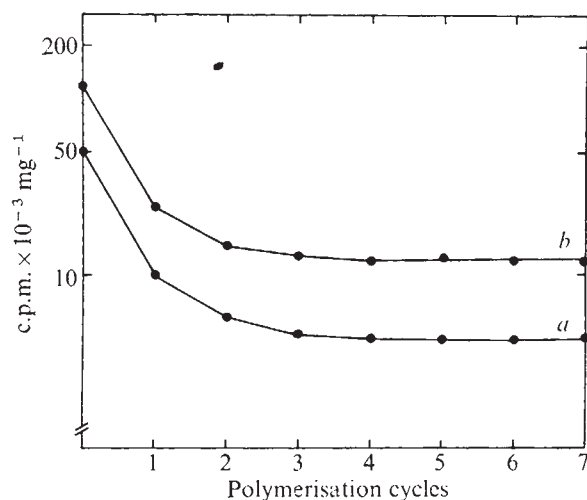


Fig. 2. SDS-disc gel electrophoretic analysis of copolymerised membrane bound tubulin with cytoplasmic rat brain tubulin. The sample analysed was from experiment 2, Fig. 1, after seven cycles of polymerisation and depolymerisation. An aliquot of 900 c.p.m. (75 µg) was electrophoresed on 5% acrylamide, 0.2% bisacrylamide and 0.1% SDS gel according to Weber and Osborne⁸ in phosphate buffer (pH 7.1). The samples were run at a current of 4 mA per tube at 23 °C until the tracking dye reached the end of the gel. The gels were stained with 0.02% Coomassie brilliant blue in 50% methanol and 9% acetic acid and destained in 5% methanol and 7.5% acetic acid. *a*, The gel was scanned at 520 nm on a Gilford spectrophotometer with a linear transport attachment; the ordinate is linear in absorbance. *b*, The gel was sliced into 1-mm disks, digested in 0.2 ml of 30% hydrogen peroxide at 37 °C for 48 h, and then counted in Aquasol.

Solubilisation of membrane tubulin using Nonidet P-40 was carried out as previously described⁶, and the soluble radioactivity was mixed with unlabelled supernatant (100,000g for 1 h) and purified through up to seven cycles of polymerisation⁷. Constant specific activity was obtained after the third cycle of polymerisation (Fig. 1). Such repolymerisation to constant specific activity is operationally equivalent to classical recrystallisation to constant specific activity and suggests strongly that the radioactivity of the membranes is either tubulin, or tau or other microtubule-associated protein.

That the radioactivity is, in fact, tubulin was verified in

Table 1 Distribution of added ³H-tubulin in different washings of crude membranes

Tissue treatment	³ H (c.p.m.)
Homogenate	12,080
Soluble supernatant	10,200
1st wash	1,100
2nd wash	200
3rd wash	25
4th wash	<5
5th wash	not detectable
6th wash	not detectable

³H-Tubulin, 12,000 c.p.m., was added to rat brain and homogenised in 3 vol membrane buffer (containing 0.25 M sucrose, 1 mM dithiothreitol, and 3 mM Tris-HCl buffer, pH 7.4). The homogenate was diluted with the same buffer to make it 10% with respect to tissue weight, centrifuged for 10 min at 700g to remove debris and the resulting supernatant solution was centrifuged at 37,000g for 10 min. The pellet was washed with membrane buffer three times and then again three times with polymerisation buffer (containing 0.1 M 2-(N-morpholine) ethane sulphonic acid (MES) (pH 6.4), 1 mM ethylene glycol bis(β-aminoethylether)-N,N'-tetraacetic acid (EGTA), 1 mM GTP, and 0.5 mM MgCl₂) before solubilisation. During the washing with polymerisation buffer, membranes were sonicated (3 s) each time to ensure complete removal of trapped cytoplasmic tubulin.

experiments where seven-cycle purified microtubules were examined by sodium dodecyl sulphate (SDS)-disc gel electrophoresis⁸. More than 90% of the protein migrated as the 55,000-dalton subunit of tubulin (Fig. 2), and all the radioactivity comigrated in this position. It seems likely, therefore, that the membrane radioactivity polymerised to constant specific activity is tubulin, and that this tubulin is normal with respect to its ability to polymerise to microtubules.

Only a small minority of electron micrographs show microtubules in close contact with plasma membranes⁹⁻¹¹, and in most cases a sizeable gap occurs between the ends of microtubules and the membrane. Similarly, freeze-fracture preparations of membranes provide no evidence for microtubules embedded in the membrane. Nevertheless, numerous models have been proposed in which microtubules directly modify membrane behaviour¹²⁻¹⁴. What then is the function of tubulin embedded in the membrane? One possibility is that it acts as a nucleation centre for microtubule polymerisation and that this region of the microtubule does not survive the usual fixation for electron microscopy. Indirect evidence that this may be so has been provided by Becker *et al.*¹⁵ who showed specific, temperature-sensitive, non-radiative energy transfer between fluorescein-labelled membranes and rhodamine-labelled tubulin. Whatever its role, the tubulin bound to membranes seems to be identical to cytoplasmic tubulin in all respects, except that it has greater thermal stability, and must be taken into account in interpreting the effects of antimetabolic drugs on membrane behaviour.

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Operon controlling motility and chemotaxis in *E. coli*

THERE has been extensive study of the genetic basis for flagellar formation^{1,2}, motility³ and chemotaxis^{4,5} in *Escherichia coli*. But in order to understand the specific mechanisms involved in these processes it is necessary to identify the gene products. We recently showed how *E. coli*- λ hybrids could be used to identify flagella-related proteins⁶ and we have subsequently isolated two phages that carry the genes directly involved in flagellar functions. One phage (Fig. 1a, λ fla2) carries *fla1*⁷ which regulates the synthesis of flagellar proteins and *mot*,

which is necessary for flagellar rotation. λ fla2 also seems to carry part of the *cheA* gene⁸, which is concerned with regulating flagellar rotation in response to chemotactic signals. It is thought to carry only part of the *cheA* gene because the phage does not show genetic complementation when it is introduced into *cheA* mutant strains. It does, however, produce wild-type recombinants with one *cheA* mutant (e14t1) but not with another (e14q1) (Fig. 1b). Furthermore, the hybrid- λ phage that carries the adjacent segment of DNA (Fig. 1a, λ fla3) also does not show complementation when it is introduced into *cheA* mutant strains. It gives wild-type recombinants with the *cheA* mutant e14q1 but not with the strain that carries the allele e14t1 (Fig. 1b). On the basis of these observations we concluded that the endonuclease (*EcoRI*) which was used to generate the DNA fragments that were cloned cleaved the *cheA* gene, and that λ fla2 carries the promoter proximal part of the gene⁸ while λ fla3 carries the distal part of the gene (Fig. 1a).

Two polypeptides FP39 (flagellar protein, molecular weight 39,000) and FP31 were synthesised after infection of ultra-violet-irradiated cells with λ fla2 and its derivatives. FP31 was identified as a product of the *mot* gene but no assignment could be made for FP39. Two possibilities were considered⁸: (1) that it was a polypeptide fragment representing the promoter proximal part of the *cheA* gene or (2) that it was the product of a second *mot* cistron which mapped between *mot* and *cheA*. To distinguish between these possibilities, a new phage was constructed that restored the continuity of the *cheA* gene. The first hypothesis predicts that the polypeptides coded for by this phage will include a new larger polypeptide replacing FP39. The second hypothesis predicts that FP39 will remain and that an additional polypeptide corresponding to the integral *cheA* gene will appear.

The steps involved in the construction of the new phage are summarised in Fig. 1a. λ fla18 was derived from λ fla2 and λ fla18 Δ 2 carried a deletion which removed the *fla1* gene and part of the *hag* gene as well as one *EcoRI* site⁸. The *mot* gene and part of the *cheA* gene (Fig. 1b) remained fused to the right arm of lambda. λ fla42 was derived from λ fla3, a deletion removed the *flaG* and *flaH* activities. λ fla42 Δ 13 carried a further deletion which removed the *cheB* gene activity as well as an *EcoRI* site leaving the other part of the *cheA* gene fused to the left arm of lambda. *EcoRI* endonuclease digestion and subsequent ligation was used to construct a new phage (λ fla52) composed of the left arm of λ fla42 Δ 13 and the right arm of λ fla18 Δ 2. The new phage carried *mot* and could form recombinants and showed complementation with *CheA*⁻ strains (Fig. 1b). Even in liquid culture *CheA*⁻ strains that showed the characteristic smooth swimming phenotype regained the ability to 'tumble' within 25 min after infection by λ fla52. Thus we conclude that λ fla52 carries the gene that can restore functional *cheA* activity.

Figure 2 shows the flagellar proteins that are synthesised after infection with hybrid λ phages. λ fla42 specifies the synthesis of the *cheB* gene products (including FP38 and FP8), the *hag* gene product, the triplet protein(s) (FP62) and a low molecular weight polypeptide (FP12). The *cheB* gene products, the "triplet" protein(s) and the low molecular weight polypeptide will be discussed in detail elsewhere. For our present purposes the "triplet" bands serve as a convenient outside marker on the left-hand part of λ fla52. Infection with λ fla42 Δ 13 results in the "triplet" and flagellin bands while λ fla18 Δ 2 gives rise to FP31 and FP39 as well as a new band FP43, which is associated with the deletion that removes flagellin activity and serves as a convenient marker for the right arm of lambda. λ fla52 specifies the synthesis of three new bands that are flagellar specific and could not have been predicted from the properties of the parent phages. The bands will be referred to as FP76, FP66 and FP12.

The restoration of the continuity of *cheA* resulted in the appearance of new polypeptides but did not affect FP39. This suggests that FP39 is not a product of the *cheA* gene but rather results from another gene between *cheA* and *mot*. Armstrong and Adler³ showed that strains carrying *mot* mutants fell into

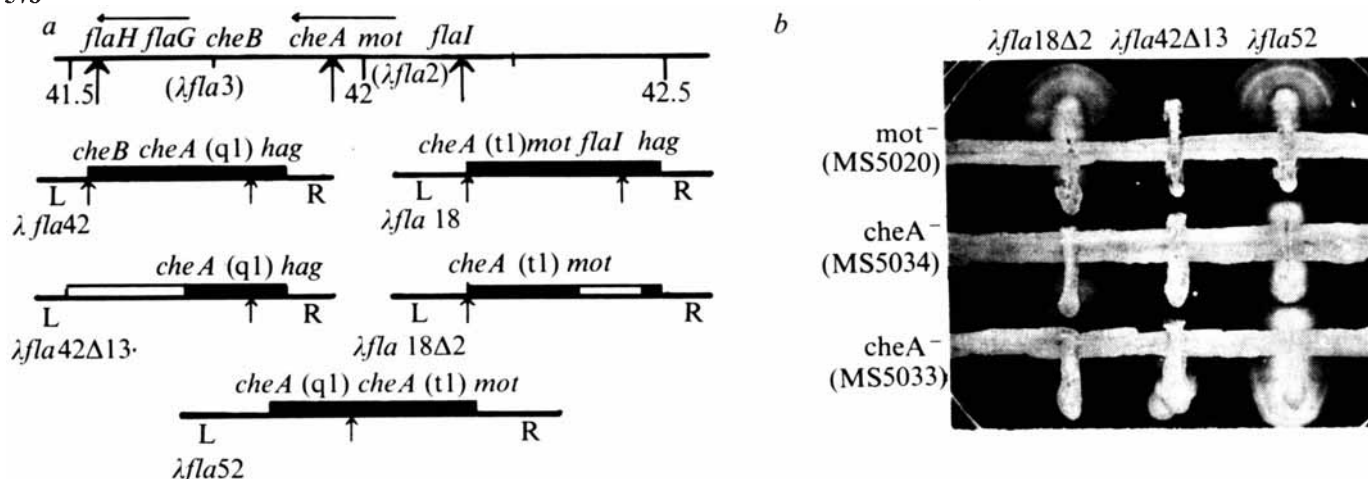


Fig. 1 *a*, Construction of λ -hybrid phage carrying intact Mocha operon. The top line shows a portion of the *E. coli* genetic map. Arrows below the line indicate the position of *EcoRI* endonuclease sites. The construction of λ *fla3*, λ *fla2*, λ *fla18*, λ *fla18 Δ 2 as well as the methods for genetic characterisation and endonuclease mapping have been described⁶⁻⁸. λ *fla42* was made from λ *fla3* Δ 1 (a deletion of *fla3* which removed *flaG* and *flaH*) and λ *fla1* Δ 4. Deletions were selected by the method of Parkinson and Huskey¹². The heavy line indicates the presence of the appropriate gene, detected by complementation or recombination. *CheA*(t1) and *CheA*(q1) refer to the ability to form recombinants with strains carrying these alleles. The light areas indicate specific deletions. λ *fla52* was constructed by mixing *EcoRI* endonuclease-treated DNA from λ *fla42* Δ 13 and λ *fla18* Δ 2 followed by annealing and ligation. After transfection 400 plaques were picked and tested. They had the genetic properties of all four possible combinations, λ *fla42* Δ 13, λ *fla18* Δ 2, λ *fla1* Δ 4 and λ *fla52*. Three plaques with identical properties were isolated: λ *fla52*, λ *fla55* and λ *fla57*. *b*, Genetic characterisation of λ *fla52* and its parent phages. The phages were streaked across tester strains⁸ carrying a *mot* mutation (MS5020, a λ lysogen of² MS797). A *cheA* mutation (MS5034, a λ lysogen of e14t1) and another *cheA* mutation (MS5033 a λ lysogen of e14q1) (both obtained from J. S. Parkinson). Recombination is recognised by the formation of swarms (diffuse spreading and concentric circles); complementation is scored by the dense short trails close to the region of infection. For example, the infection of MS5020 by λ *fla18* Δ 2 shows both recombination and complementation, MS5034 shows only recombination and MS5033 shows neither.*

two groups, *motA* and *motB*. They could not, however, exclude the interpretation that these represented intracistronic complementation within a single *mot* gene. In *Salmonella*⁹ there are apparently two cistrons, *motA* and *motB*. We tested a large number of deletions of the phage for complementation and recombination with strains carrying *motA*, *motB* and *cheA* mutations. Figure 3 shows that the deletions of λ *fla52* could

distinguish clearly between *motA* and *motB* mutations. Furthermore, with the exception of λ *fla52* Δ 7 (which probably represents a deletion within the *motA* structural gene) deletions into *motA* (λ *fla55* Δ 1) resulted in loss of expression of *motB* and *cheA* function, although deletions into *cheA* or *motB* had no effect on *motA*. This kind of polarity can best be interpreted in terms of an operon in which the order of transcription is

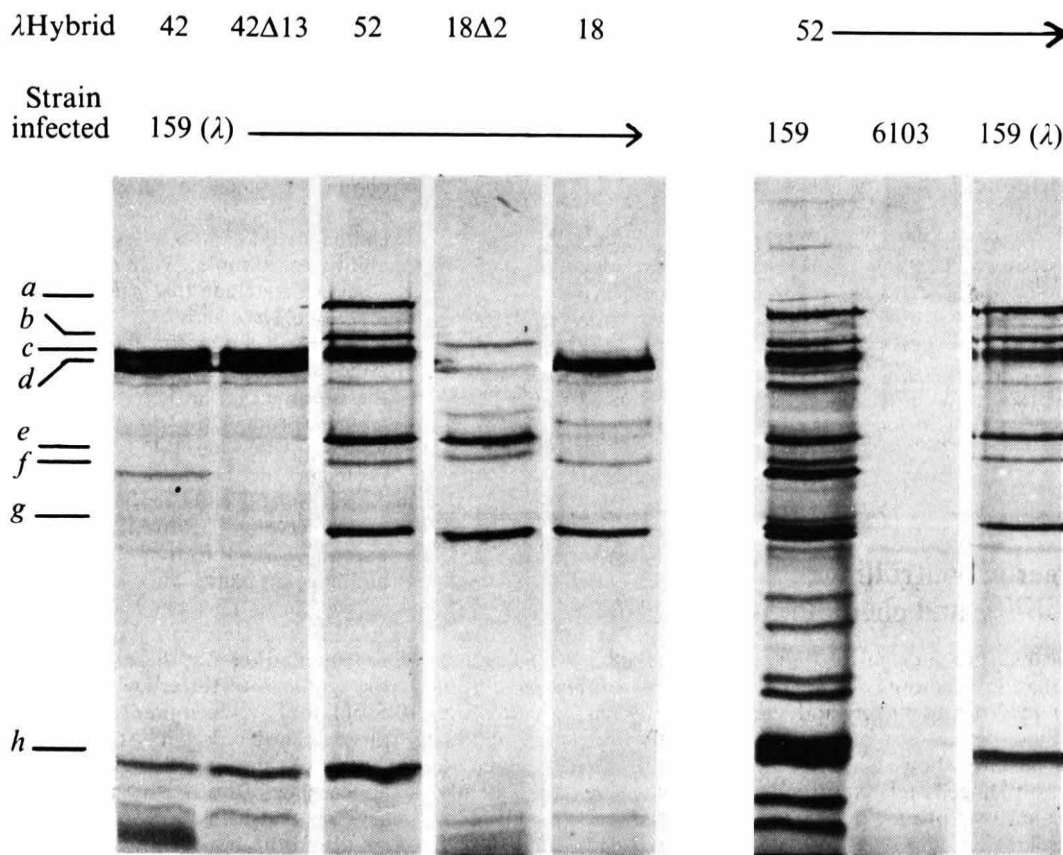


Fig. 2 Electrophoresis of proteins synthesised in ultraviolet-irradiated cells infected with hybrid λ . Conditions of infection, labelling electrophoresis and autoradiography have all been described⁶⁻¹³. The letters on the left of the figure refer to the bands on the gel: *a*, FP76; *b*, FP66; *c*, triplet, FP62; *d*, flagellin; *e*, FP39; *f*, FP38; *g*, FP31; *h*, FP12. Infection of strain 159 with λ *fla52* results in the expression of proteins coded for by λ as well as those specified by λ *fla52*. Strain 6103 carries the *flaI* mutation and is also a lysogen. The *flaI* mutation specifically represses the synthesis of flagellar gene products and the resident λ represses λ -specific gene products¹³.

motA, *motB* and *cheA*⁸. We refer to this grouping as the Mocha operon. The "triplet" product is not included in the Mocha operon.

Further evidence for an operon-like unit comes from observations on the effects of the deleted phages on the behaviour of *mu*-induced *mot* mutants. When the *mu* genome is inserted in the *motA* gene it eliminates both motility and chemotaxis (J. S. Parkinson, personal communication). When λ *fla52Δ1* (*MotA*⁻ *MotB*⁺ and *CheA*⁺) infected the *mu mot* mutants it restored both motility and chemotactic behaviour (tumbles) in less than 30 min. λ *fla57Δ5* (*motA*⁺, *motB*⁺) restored motility but the cells showed the smooth swimming phenotype characteristic of *CheA*⁻ mutant strains. Neither λ *fla52Δ7* nor λ *fla52Δ11* restored motility, thus both the *motA* and *motB* products are necessary for motility.

The polypeptides that are determined by the deleted phages are shown in Fig. 3. Band FP31 corresponds to *motA*, FP39 corresponds to *motB* and FP76, FP66 and FP12 all correspond to *cheA*. The three *cheA* polypeptides could result from processing of a single polypeptide or alternatively there may be three contiguous genes that code for these polypeptides. In order to distinguish between these possibilities heteroduplexes between λ *fla52Δ7* DNA and λ *fla52Δ1* DNA were formed. λ *fla52Δ1* deletes the triplet band which should map on the left side of the *cheA* gene and should be contiguous with the longer left arm of lambda. In λ *fla52Δ7* only the *motA* activity is deleted, which suggests a small deletion on the right side of the

Fig. 3 Correlation between genetic properties and specific protein synthesis. The table on the top summarises the results of complementation and recombination tests: +, complementation and recombination; R, recombination alone; -, neither. The strains MS5037 (a λ lysogen of strain 448 *motA*), MS5038 (a lysogen of 507 *motB*) and MS5039 (λ lysogen of 483 *motB*) (all obtained from J. Adler) were originally isolated and characterised by Armstrong and Adler³. The acrylamide gel bands are referred to in the same way as in Fig. 2. The intense band above the one labelled *e*, is FP43.

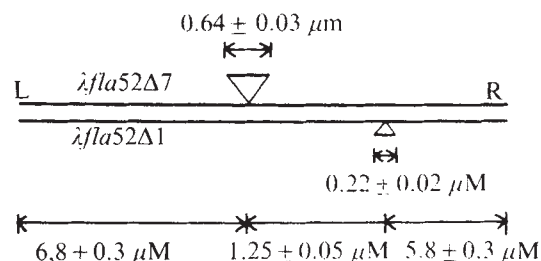
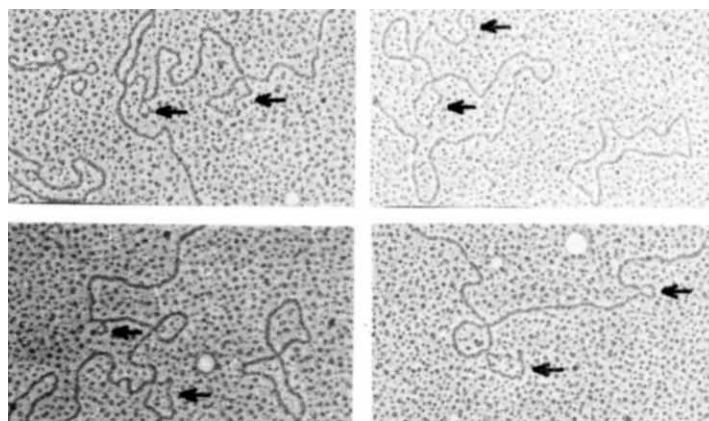
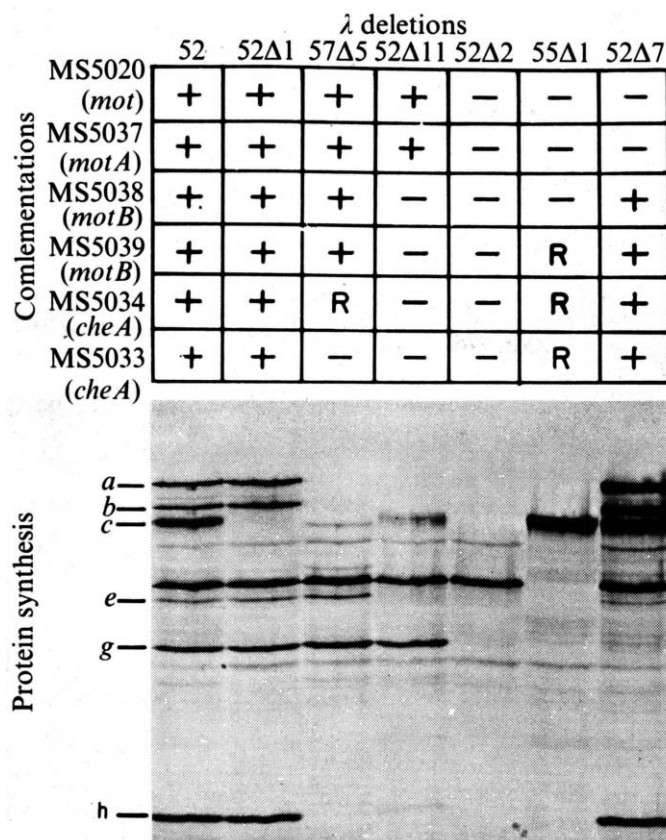


Fig. 4 Heteroduplex maps of λ *fla52Δ7* and λ *fla52Δ1* DNA. Heteroduplex mapping was done according to the procedure of Davis¹⁴; 25 molecules carrying the double loops were photographed in the presence of circular colicin F1 DNA (2.14 μM). The photographs were traced and the relative contours calculated with a Hewlett Packard calculator digitiser. The col F1 DNA was used as reference.

motB gene contiguous with the shorter right arm of lambda. The region between these two deletions corresponds to the *motB* and *cheA* genes. Twenty-five heteroduplex molecules with double loops were measured; the results are shown in Fig. 4. The distance between the deletion loops corresponds to 8.5% of lambda, or to 3.7 kb pairs, or to 1,230 amino acids. If all of the DNA is transcribed and translated into protein, this must account for both the *motB* and *cheA* polypeptides. Thus the *cheA* gene product could not have more than 835 amino acids. This is slightly more than half the number of amino acids required to make up three polypeptides with apparent molecular weights of 76,000, 66,000 and 12,000.

We conclude that the *cheA* gene is part of a cotranscribed unit Mocha. The gene products resulting from this operon include proteins of molecular weight 31,000 and 39,000 that correspond to the *motA* and *motB* cistrons and three polypeptides of molecular weight 76,000, 66,000 and 12,000 that correspond to the *cheA* region of the genome. This region does not have the contiguous coding capacity sufficient to account for unique polypeptides of these sizes. There are various mechanisms that could rationalise this apparent discrepancy, for example, modification or processing of a single precursor polypeptide, or modifications in the transcription or translation processes¹⁰. Analysis of the peptide maps should distinguish between these possibilities.

In any event, if all three polypeptides have a function in chemotaxis, it may help to explain the complexity in the complementation patterns observed with *che* mutants in *E. coli*⁵ and *Salmonella*¹¹.

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Ends of bacteriophage Mu DNA

THE temperate bacteriophage Mu does not prefer a specific attachment site on the chromosome of *Escherichia coli* and can apparently engineer the insertion of its DNA irrespective of the host sequences encountered¹. The highly promiscuous, non-homologous integrative recombination between Mu DNA and *E. coli* DNA has prompted a comprehensive analysis of the structure of the Mu genome. The DNA molecules in mature Mu virus particles are linear duplexes of 37-38 kilobases (kb) (ref. 2). When the two Mu DNA strands are separated and reannealed, complete duplexes are not regenerated. Instead, one end is always split into two single-stranded tails, which can be readily seen with an electron microscope^{3,4}. This end of Mu DNA has been identified as the right end or the S end⁵. The single-stranded tails are generally 1.5 kb in length, although occasionally the single strand can be as short as 0.5 kb or as long as 3.0 kb (ref. 6). The non-renaturation of the S end clearly reflects the heterogeneity of the terminal sequences. Studies on the renaturation kinetics of the Mu DNA have implied that *E. coli* sequences at the S end are responsible for the heterogeneity⁷. Allet and Bukhari have proposed that the left end of Mu DNA, the c end, is also not fixed and varies in length by about 100 base pairs⁸. This proposal stemmed from the observation that the DNA fragment cleaved from the c end of Mu by the restriction endonuclease *Hind*III (from *Hemophilus influenzae*) does not give a sharp band on polyacrylamide or Agarose gels after electrophoresis. The heterogeneity of the c end is normally not detectable by electron microscopic techniques.

Bukhari and Taylor have presented evidence showing that the heterogeneity at the S end is a consequence of the headful packaging of Mu DNA from maturation precursors which have *E. coli* DNA covalently linked to the phage DNA⁶. The packaging reaction starts in an oriented manner from the c end and proceeds toward the S end. This inference is supported by partial denaturation mapping studies of Inman *et al.*⁹ They have demonstrated that the S end of Mu is situated at the phage head-tail junction. We report here experiments which demonstrate conclusively that *E. coli* DNA is present at the c end as well as the S end of Mu. We further show that the end sequences represent different parts of the *E. coli* chromosome and that the amount of host sequences at the c end is about tenfold less than those present at the S end.

We hybridised radioactively labelled *E. coli* DNA to the Mu DNA fragments generated by various restriction endonucleases. These experiments were based on the technique

of Southern¹⁰, in which DNA fragments from the Agarose gels are transferred to a nitrocellulose paper and then hybridised with the radioactive RNA or DNA probe. Autoradiography then reveals which, if any, fragments give significant specific hybridisation with the probe. If Mu contains *E. coli* DNA at the ends, only the two terminal fragments would hybridise with the *E. coli* DNA whereas the internal fragments would not.

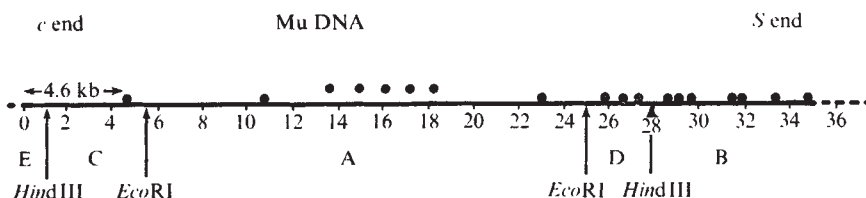
We cleaved Mu DNA with the restriction endonucleases *Hind*II and *Hind*III and *Eco*RI and separated the resulting fragments by electrophoresis in Agarose gels. The sites at which these enzymes cut Mu DNA are shown in Fig. 1 (ref. 8). Both *Hind*III and *Eco*RI are known to cleave Mu DNA at two sites. *Hind*III gives a diffuse fragment of about 1.1 kb from the c end and a fragment of about 7 kb from the S end. *Eco*RI produces a fragment of about 5.1 kb from the c end and of about 10 kb from the S end. *Hind*II cleaves Mu DNA at 17 sites, the c end fragment being 4.5 kb in length. No S end fragment could be seen, however, with *Hind*II. Presumably *Hind*II cuts too close to, and within, the heterogeneous sequences resulting in a whole range of different size fragments.

The hybridisation of ³²P-labelled *E. coli* DNA to Mu DNA fragments generated by *Hind*II is shown in Fig. 2. The fragments were first stained with ethidium bromide, photographed under ultraviolet light and then transferred to a nitrocellulose paper for hybridisation. It can be seen in the autoradiograph that only one Mu fragment hybridised with the *E. coli* DNA. We know that this fragment, about 4.5 kb in length, comes from the c end² and as expected is further cleaved by *Hind*III. To show the presence of host sequences at the S end, the fragments generated by *Hind*III and by the combined action of *Hind*III and *Eco*RI were hybridised with ³²P-labelled *E. coli* DNA. As shown in Fig. 3, the fragment representing the S end as well as the fragment representing the c end specifically hybridised with *E. coli* DNA, whereas the other fragments did not.

Because of the heterogeneity of the Mu DNA ends, it is logical to assume that the host sequences do not arise from a specific segment of the host chromosome but that they are derived from all parts of the host genome. To test this assumption, *E. coli* DNA was cut with *Eco*RI or *Hind*III and the fragments were hybridised with ³²P-labelled Mu DNA. Figure 4 shows that whereas calf thymus DNA showed no hybridisation with Mu DNA, all *E. coli* DNA fragments picked up the ³²P label. The general hybridisation could be seen particularly with the larger fragments of *E. coli* DNA in the upper part of the gel. Hybridisation of *Eco*RI fragments of *E. coli* with the purified ³²P-labelled S end and the c end fragments provides results similar to the whole Mu DNA. It can be inferred therefore that all parts of the *E. coli* genome are represented at the ends of Mu DNA. Whether all parts have an equal chance of being present at the end cannot be determined precisely at this time. It can be seen in Fig. 5 that some host fragments stand out more clearly above the general hybridisation background. It is not clear whether these host segments are overrepresented at the ends or whether Mu DNA also has some specific sequences related to sequences in *E. coli*.

The relative amounts of host DNA at the c end and at

Fig. 1 Cleavage map of Mu DNA. Mu DNA is drawn as a solid line calibrated in kilobases. The broken line at the end represents host DNA. The sites at which *Hind*II cleaves Mu DNA are shown by solid circles. The circles above the line indicate *Hind*III sites, in the middle of Mu DNA, which have not been ordered with respect to each other. The c end fragment produced by *Hind*II is shown as 4.6 kb long segment. Cleavage sites of *Hind*III and *Eco*RI are shown by arrows. A-E, Fragments generated in the presence of both *Hind*III and *Eco*RI.



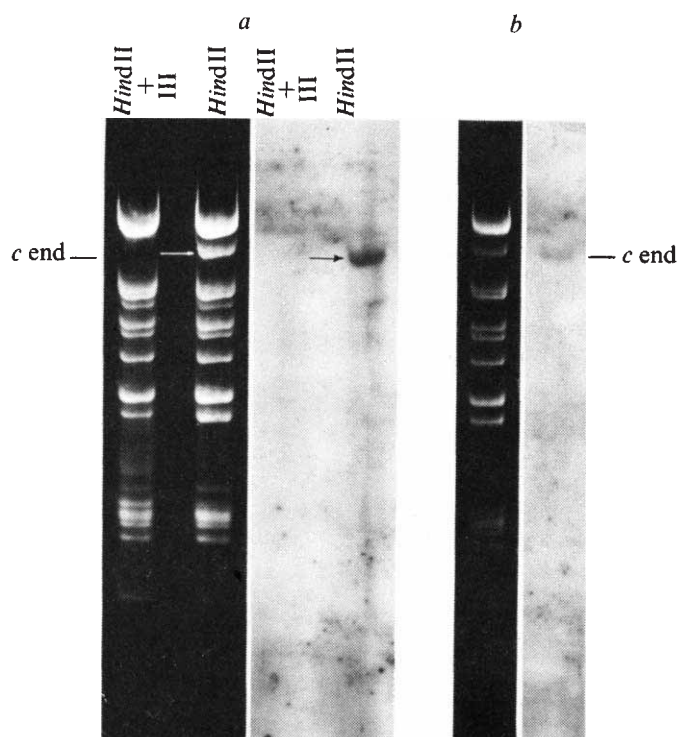


Fig. 2 Hybridisation with *E. coli* DNA to Mu DNA fragments generated by *Hind*II and *Hind*II + *Hind*III. Bacteriophage Mu particles were purified by caesium chloride density gradient centrifugation; the DNA was extracted with phenol and digested with *Hind*II and *Hind*II + *Hind*III (refs. 6 and 8). The fragments were separated by electrophoresis in 1.4% Agarose slab gels, stained with ethidium bromide and photographed under ultraviolet light, as described by Sharp *et al.*¹¹. To denature the fragments, the gels were soaked in 0.2 M NaOH, 0.6 M NaCl for 40 min and this solution was then replaced by 0.6 M NaCl and 1 M Tris, pH 7.4, for 30 min. After denaturation and neutralisation the fragments were transferred to nitrocellulose paper (Millipore 25 HAWP) as described by Southern¹⁰. The gel strip containing the denatured fragments was placed on a large sheet of Whatman MM paper, wetted with 4 × SSC (SSC = 0.15 M NaCl, 0.15 M Na citrate), on a glass plate. A strip of nitrocellulose paper, a strip of Whatman MM paper and some absorbent papers, in that order, were laid on top of the gel strip and the large sheet of Whatman paper was continuously wetted with 4 × SSC, with frequent changes of the absorbent papers. The DNA became trapped in the nitrocellulose paper as 4 × SSC passed through the gel, drawn by the filter paper¹⁰. After the transfer of the fragments was complete (about 3 h), the nitrocellulose paper was dried, baked in an oven at 65 °C and soaked for a few hours in Denhardt's solution¹² to reduce nonspecific binding of labelled DNA. The hybridisation with denatured ³²P-labelled *E. coli* DNA was then carried out using the standard method of DNA-DNA hybridisation on filters¹². The ³²P-labelled *E. coli* DNA was prepared by growing *E. coli* K12 cells in a low phosphate medium, supplemented with H₂³²PO₄. The hybridisation mixture contained 0.1–0.5 mg ³²P-labelled denatured probe DNA (generally with 2–5 × 10⁵ c.p.m.), 0.5% SDS, in 1 × Denhardt's solution and 6 × SSC. After hybridisation the nitrocellulose paper was washed extensively in 2 × SSC + 0.5% SDS at 60 °C, dried and autoradiographed. For further technical details see ref. 13. The figure shows Mu DNA fragments visualised after ethidium bromide staining (left hand columns) and after hybridisation with ³²P-labelled *E. coli* DNA (right hand columns) Mu *crs*62(a) was grown by induction of strain BU 165 which contains a single copy of Mu *crs*62 mom⁶. Mu *c*3(b) was grown by infection. The position of the *c* end fragment with *Hind*II is indicated by an arrow. This fragment is cut by *Hind*III and thus does not appear in the *Hind*II + *Hind*III digest of Mu *crs*62 DNA. The *S* end fragment is too heterogeneous to be seen in these conditions⁶. Hybridisation with the *c* end fragment is visible in the autoradiograph. Hybridisation with the diffuse *c* end fragment generated by *Hind*III (labelled *Hind*IIIc) is too low to be detected here and is shown in Fig. 4.

the *S* end could be determined by hybridising Mu fragments with varying concentrations of *E. coli* DNA. The *c* end and the *S* end bands were cut out from the nitrocellulose papers after hybridisation and read in a scintillation counter. *E. coli* DNA always hybridised with the *S* end 10–20-fold more than with the *c* end. Since the mean length of the *S* end heterogeneous sequences is about 1.5 kb (see ref. 6), it follows that the *c* end generally has 75–150 base pairs of heterogeneous host sequences.

Fig. 3 Hybridisation with *E. coli* DNA to Mu fragments produced by *Hind*III, *Eco*RI and *Hind*III + *Eco*RI. Mu *c*3 DNA fragments visualised after ethidium bromide staining are on the left. Autoradiograph of the same fragments after hybridisation with ³²P-labelled *E. coli* DNA is on the right. The experimental procedure was as in Fig. 2 except that electrophoresis was carried out in 0.9% Agarose gels to resolve large fragments. In each case the most rapidly moving band represents the *c* end and the second band from the top represents the *S* end. In *Eco*RI digest a partial fragment, because of incomplete digestion, can be seen at the top. The marker DNA was a mixture of labelled Mu fragments and an unrelated labelled fragment. The unrelated marker fragment can be seen as a dark band in the autoradiograph. The fragments and their pattern of hybridisation are diagrammatically shown in the insets at the bottom. With *Hind*III and *Eco*RI, the middle fragment of Mu did not show significant hybridisation with *E. coli* DNA, whereas both the *c* end and the *S* end fragments did. With *Hind*III + *Eco*RI digestion, out of 5 fragments (see map in Fig. 2) only B (*S* end) and E (*c* end) hybridised with *E. coli* DNA.

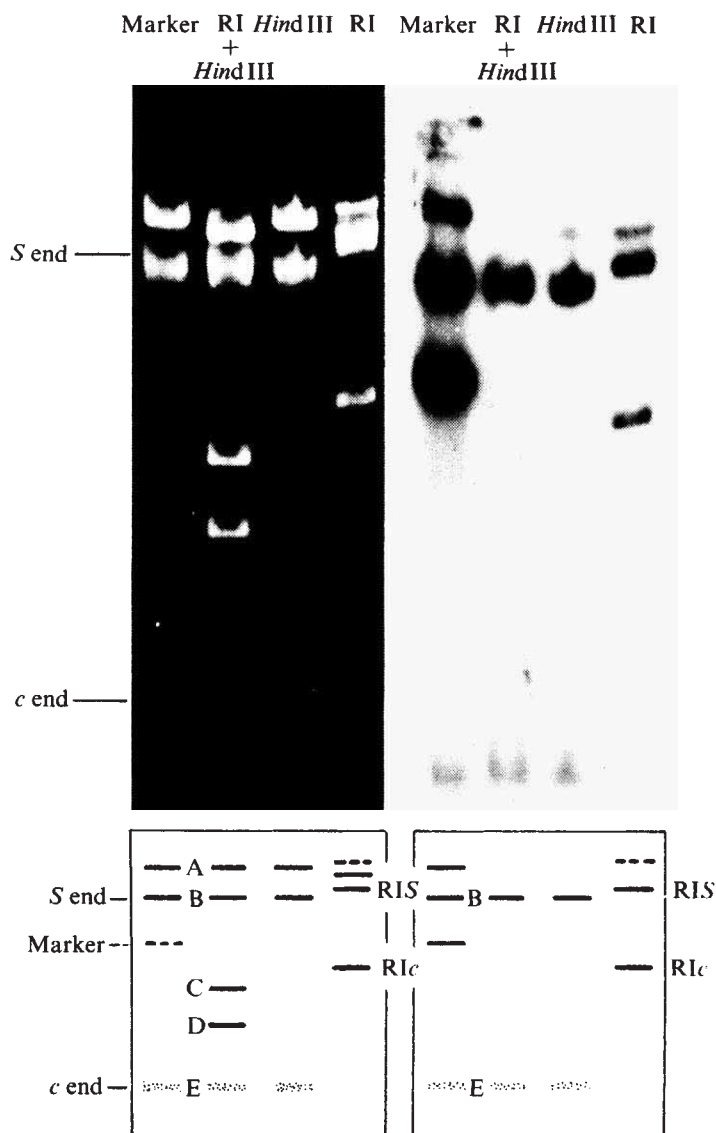
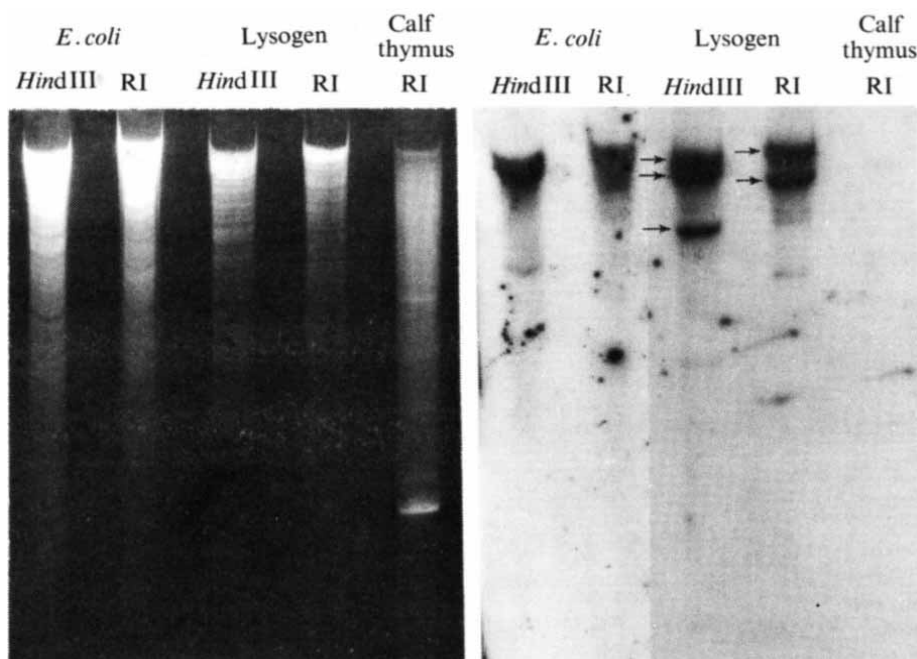


Fig. 4 Hybridisation with Mu DNA to fragments of *E. coli* DNA. Total cellular DNA from KL16, an *E. coli* K12 strain, and from a Mu *cts62* lysogen was extracted three times with phenol and digested with *Hind*III or *Eco*RI. Calf thymus DNA, digested with *Eco*RI, was used as control to check for stringency of hybridisation. Mu DNA was labelled *in vitro* with ^{32}P to high specific activity by the so-called "nick translation technique", as described by Maniatis *et al.*¹⁵. DNase I (1 ng) was added to cause nicks and then the nick translation reaction carried out with DNA polymerase using ^{32}P -labelled nucleotide triphosphates. The DNA bands visualised after ethidium bromide staining are on the left. The brightly stained bands in the calf thymus DNA column are comprised of tandemly-repeated sequences that are represented many times in the DNA complement. No hybridisation of Mu DNA with calf thymus DNA can be seen in the autoradiograph on the right. Hybridisation with *E. coli* DNA can be clearly seen at the top; the intensity of bands in the autoradiograph decreases as fragments become smaller and smaller towards the bottom of the gel. The *Hind*III digest of DNA from a lysogen (BU 1209) shows three prominent bands whereas the *Eco*RI digest of the lysogen DNA shows two very strong bands after hybridisation. These bands, indicated by arrows, represent the prophage Mu DNA. The principle for detecting the integrated Mu genome was as described by Botchan *et al.* for determining the organisation of the simian virus 40 DNA in rat cells transformed by SV40. In the *Eco*RI digest of the lysogen DNA, the top band represents the middle part of the prophage genome and the *S* end junction fragment of prophage and host DNA. The second band represents the *c* end junction fragment. In the *Hind*III digest of the lysogen DNA the top two bands (not completely resolved) represent the middle fragment and the *S* end junction fragment. The third band is the *c* end junction fragment. The size of the end fragments has changed because of fusion with host DNA. The significance of the minor discrete bands in *E. coli* DNA digests, which can be seen after hybridisation with ^{32}P -labelled Mu DNA, remains unclear.



These results provide direct proof that *E. coli* sequences are present at both ends of Mu DNA and that these sequences are picked up from different parts of the chromosome of *E. coli*. The host sequences at the *c* end correspond to 75–150 base pairs. These sequences, as opposed to the *S* end sequences, are generally too short to be seen by electron microscopy of denatured and renatured Mu DNA molecules. It is possible, however, to see them electron microscopically by binding T4 gene 32 protein to the single strands (H. Delius, personal communication).

The host sequences at the *S* end have been postulated to arise by headful packaging of Mu DNA from maturation precursors in which Mu DNA is covalently linked to host DNA. This would mean that the headful packaging of Mu

starts from the *c* end, linked to host DNA, and proceeds toward the *S* end, also linked to host DNA and that the presence of host DNA at the *S* end is a phenomenon of the size of Mu DNA. Experimental evidence has confirmed that insertions in Mu DNA cause a reduction in the length of the *S* end host sequences, as expected from the headful packaging model.

The *X* mutants of Mu, containing an ISI type of insertion of about 800 base pairs¹⁴, show shorter single-stranded tails on denaturation and renaturation⁶. Insertion of a 2.8 kb long translocation element, carrying gene for chloramphenicol resistance, completely eliminates the *E. coli* sequences at the *S* end (Chow and A.I.B., unpublished). Insertions in Mu seems, however, to have no effect on the

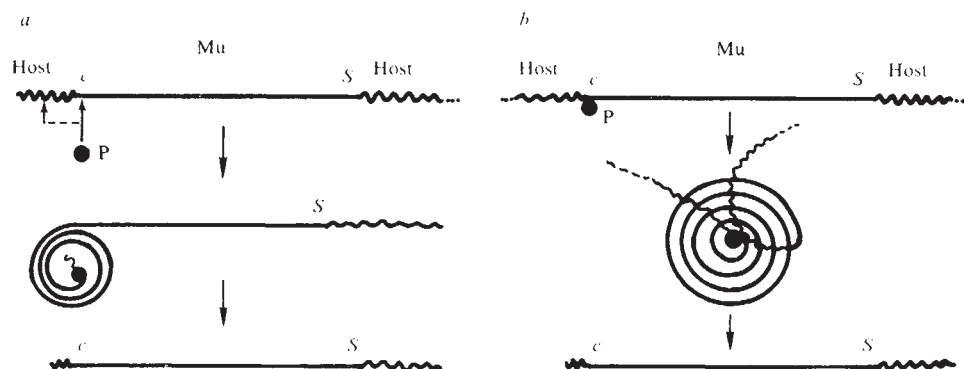


Fig. 5 Generation of *E. coli* DNA at the ends of Mu. Two possible models shown are based on the assumption that the maturation precursor contains *E. coli* DNA linked to both ends of Mu DNA. *a*, P recognises a specific site at the *c* end but cuts to the left of the prophage DNA, into the host DNA. The DNA is then folded until a headful is reached at the *S* end. *b*, P recognises a specific site at the *c* end and serves as a nucleation centre for condensing DNA. When a headful is reached, cuts in DNA are made. The cut into *E. coli* DNA near the *c* end is made because the nucleation centre is large, covering a part of the host DNA at the *c* end. The representation is strictly diagrammatical.

size of the host sequences at the *c* end. The *c* end fragment produced by *Hind*III remains diffuse on gels in the case of both small and large insertions in Mu DNA (data not shown). The acquisition of 75–150 host base pairs at the *c* end is apparently a specific event, unrelated to the size of Mu DNA being packaged. Two possible ways in which a small amount of host DNA can be linked to the *c* end of viral DNA are depicted in Fig. 5. It can be postulated that the 'packaging proteins' recognise a specific site, very close to the *c* end of the Mu DNA, cut the DNA to the left of the recognition site and then fold the DNA until a certain size is reached. This would imply an interesting endonucleolytic cut by Mu proteins. Alternatively, the DNA could be condensed by a large packaging complex which covers a part of the host sequences linked to the *c* end such that when the packaged DNA is cleaved from the maturation precursor some host DNA is retained at the end. In any case, cutting into host sequences at the left, and also to the right, of the actual Mu DNA ends ensures that Mu sequences remain intact during the viral life cycle.

Mu is the only viral system known so far in which viral DNA acquires new host sequences during maturation. The normal presence of host DNA at phage ends thus has no precedent. The temperate bacteriophages generally have either cohesive ends or have duplicated end sequences which are used to efficiently convert the linear DNA molecules into circular forms. Bacteriophage Mu falls into a third category. It has heterogeneous sequences at the ends of its DNA and has no obvious means of fusing them to form circular molecules.

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Table 1 Photoformation of PCBs from chlorinated benzenes*

Chlorinated benzenes	0	Irradiation (d)			No. of Cl atoms calculated
		14	28	56	
MCB	5.5	690	1,060	NA†	1
<i>o</i> -DCB	1.4	940	970	2,270	3
<i>m</i> -DCB	1.1	340	350	520	3
<i>p</i> -DCB	0.7	310	340	1,860	3
1,2,3-TCB	0.7	29	32	32	5
1,2,4-TCB	4.0	5,710	8,470	9,770	5
1,3,5-TCB	1.7	56	98	160	5
1,2,3,4-TECB	2.4	680	1,200	4,280	7
1,2,4,5-TECB	4.0	9	26	NA	7
HCB	1.0	36	41	64	5

*p.p.m.

†NA, not analysed.

materials were purchased (Wako Pure Chemical Industries, Ltd, Osaka, Japan); monochlorobenzene (MCB), *o*-, *m*-, *p*-dichlorobenzene (DCB), 1,2,3-, 1,2,4-, and 1,3,5-trichlorobenzene (TCB), 1,2,3,4-, and 1,2,4,5-tetrachlorobenzene (TECB), and hexachlorobenzene (HCB). Twenty grams of each of these were placed in 100-ml Hario brand borosilicate glass-stoppered Erlenmeyer flasks (Shibata Chemical Apparatus Co., Tokyo) which were stoppered and irradiated outdoors in sunlight for 56 summer days in Kochi, Japan.

The separation of PCBs from chlorobenzenes was carried out as follows: 0.1 g of an irradiated chlorobenzene was dissolved in 2 ml hexane, placed on a 20-g, 19-mm interior diameter silica gel column and eluted first with 250 ml hexane which removed MCB, DCBs, TCBs, TECBs and HCB, and then with 300 ml methylene chloride–hexane–acetonitrile (80:19:1) which eluted PCBs. The second fraction was cleaned up with a 10-g Florisil column using 200 ml hexane as eluting solvent. Average recoveries of PCBs in this separation process were 96.9% when the sample has fortified at 100 p.p.m. with KC-500, an industrial PCB.

The quantitation of PCB residues has usually been done by measuring the total peak height of the electron capture GLC response for residue against that of industrial PCB such as KC-500. We did not determine PCBs formed from chlorinated benzenes by the above method because their GLC profiles were not similar to those of industrial PCB. The fraction containing PCBs was perchlorinated using the method described previously^{7,8}. Decachlorobiphenyl formed by perchlorination of a mixture of PCBs was easily detectable as a single peak by electron capture GLC using 1% SE-30 column at 210 °C. The residue values were determined based on the electron-capture response to the decachlorobiphenyl compared with the standard, and then calculated so as to express the amount of PCBs containing an equivalent number of chlorine atoms.

PCBs were determined in all irradiated chlorobenzenes throughout the experimental period. GLCs showing PCB photoformation from a number of chlorinated benzenes are presented in Fig. 1, and the amounts formed are given in Table 1. A single peak due to a substance containing one chlorine atom was found to originate from MCB; PCBs containing three chlorine atoms were photoformed from DCBs; PCBs formed from TCBs almost always contained five chlorine atoms (at least six PCBs were observed in products from 1,2,4-TCB compared with only one PCB formed from 1,2,3-, and 1,3,5-TCB). Several PCBs containing seven chlorine atoms were formed from each of the TECBs; PCBs formed from HCB were found to have four or five chlorine atoms; GLC–MS suggested that the chemical structure was dimethyl chlorobiphenyl but it is unclear where the methyl groups originated; the problem is under investigation.

The reaction mechanism of photoformation of PCBs may

Photoformation of polychlorinated biphenyls from chlorinated benzenes

In recent years there has been considerable public concern about polychlorinated biphenyl (PCB) residues in the environment. Many studies have indicated the main causes and the present problems with regard to the contamination by PCBs^{1–6}. Little attention has, however, been given to the possible presence of PCBs in many organic chemicals. We report here the formation of PCBs from some chlorinated benzene compounds by sunlight irradiation, and show that these PCBs have different gas–liquid chromatographic (GLC) profiles from the industrial PCBs.

The following reagent grade chlorobenzenes used as

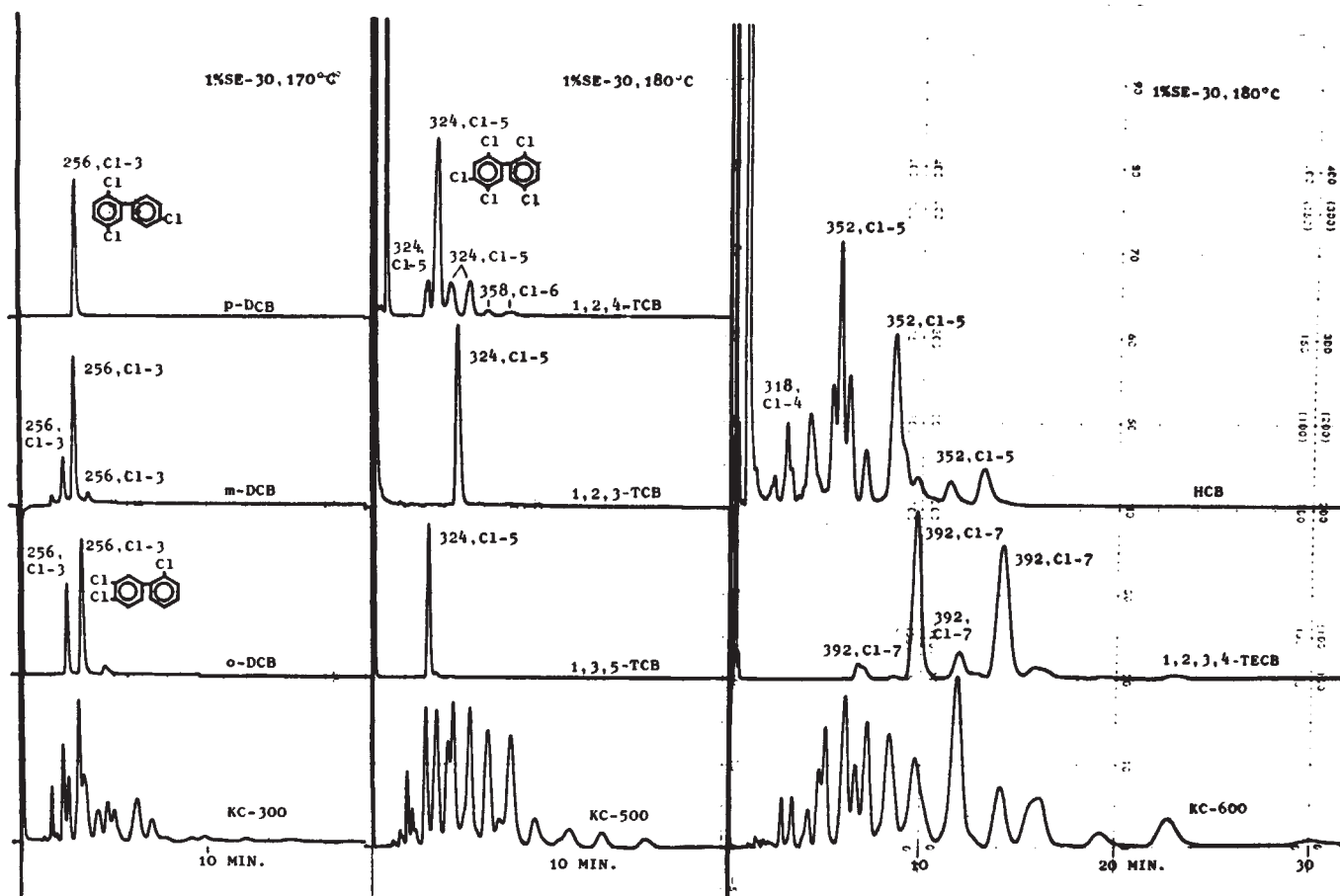


Fig. 1 Electron capture gas-liquid chromatograms of PCBs formed from chlorinated benzenes by sunlight irradiation. The KC series are Kanechlor, industrial PCBs (Kaneagafuchi Chemical Industries Co, Ltd, Osaka). Each peak was determined by GLC-MS and both the GLC peak position and the *m/e* of the molecular ion are shown together with the chlorine content. The chemical structure of substances causing the main peaks of PCBs have been proposed by comparison with GLC reference standard of known chlorinated biphenyls made by Analabs Inc., Connecticut.

involve free radical reactions based on the dehydrochlorination of one molecule from two molecules of the parent chlorobenzene. This is supported by the following: (1) the formation of hydrochloric acid in the reaction mixture; (2) the number of chlorine atoms in PCBs formed by irradiation was one less than those contained in two molecules of the equivalent parent chlorobenzene; (3) PCB formation was remarkably inhibited when the radical scavenger, DPPH(2,2-diphenyl-1-picrylhydrazyl) was added to a chlorobenzene, and the amount of PCB formed decreased to approximately one hundredth in the case of 1,2,4-TCB. Conversely, an increase in photoformation resulted when photosensitizer, benzoyl peroxide, was added, the amount of PCB formed increasing threefold in the case of 1,2,4-TCB; (4) in addition, it is possible to deduce theoretically that 2,5,4'-trichlorobiphenyl is formed from *p*-DCB and 2,4,5,2',5'-pentachlorobiphenyl from 1,2,4-TCB.

Although each chlorinated benzene has strong ultraviolet absorption in the ranges 210–230 nm and 250–300 nm, the borosilicate glass container prevented sunlight at wavelengths less than 300 nm from entering. Therefore, it was impossible to investigate the possible effect of the ultraviolet component of sunlight in these experiments, but PCB formation from chlorinated benzenes under irradiation by ultraviolet light is now being studied.

Previous workers^{9,10} have indicated that some PCBs are products of DDT photolysis. This paper demonstrates that appreciable quantities of PCBs are also formed photo-

chemically from other types of synthetic organochlorine compounds. We suggest that caution should be exercised when manipulating chlorinated benzenes industrially and when handling household insecticides such as *p*-dichlorobenzene.

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Christmas books supplement

Colour, song and movement

Peter Conder

BIRDS probably command a greater following at many levels of interest throughout the world than any other group of wild animals or plants because they are easily seen and recognised. Their colours, songs and movements fascinate, and their ways of life provide an interest for a growing number of serious bird-watchers. The books reviewed here reflect some of the different levels and areas of interest in birds: one relates facts about the adaptations of birds to their habitats in various parts of the world; and five others deal with more discrete groups. None of the books is highly technical.

I tend to be suspicious of large 'coffee-table' books which consist of glossy photographs or paintings often supporting a poor text. In *Birds and their World* (Hamlyn: London and New York, £2.95), however, John Andrews has written one of the best texts that I have found in such surroundings. Lucidly written, and full of facts about the relationship of birds to their habitat in the major biomes of the world, the book describes structural and functional adaptations to the particular characteristics of the biomes. A single chapter on migration seems out of place. As one might expect, since it is a Hamlyn publication, three-quarters of the book are taken up by colour photographs. These are of ex-

cellent quality generally, but a few have been enlarged far too much. At £3.95 it is good value for the general bird-watcher.

More expensive and less value for money is *Penguins: Past and Present, Here and There* (Yale University: New Haven and London, £6) by George Gaylord Simpson, Professor of Geosciences at the University of Arizona. Simpson became interested in penguins when, in the course of field research, he discovered a collection of fossil penguin bones. Despairing of finding any palaeo-ornithologist to work on the collection, he embarked on the task himself. In his book, therefore, the most interesting and worthwhile chapter is written round his own discoveries. Since that study he has visited many penguin colonies but has not been able to undertake further research. The remainder of this book therefore relies on previously published literature, of which he gives a limited bibliography. It is a pleasant enough "informal account" to quote the book jacket. The black-and-white photographs have not reproduced too well and the colour photographs are amateurish.

Very different is the contribution of the author in two books on raptors—*Birds of Prey: Their Biology and Ecology* (Hamlyn: London and New York, £4.50) and *Eagles of the World* (David and Charles: Newton Abbot and London, £4.95). Nominally they are put out by two different publishers but the page design, type and binding are

extraordinarily similar. In both books Leslie Brown reviews what is known about various aspects of the life of raptors—classification and distribution, habitats, way of life, way of hunting, breeding biology, ecology—and adds much original material. There is some overlap between the books, and in *Birds of Prey*, the author inevitably writes in greater detail on eagles. In both books he points out areas in which research is needed and could be easily undertaken. The books are rather discursive in style but interesting in an anecdotal sort of way. Both books are illustrated by explicit diagrams and line drawings; black-and-white photographs only in *Eagles of the World*, but both colour and black-and-white photographs in *Birds of Prey*. Each is provided with a fairly large "select" bibliography, very useful appendices summarising a variety of data. Both are good value for money.

Winter Birds (Michael Joseph: London, £5.75) by Malcolm Ogilvie concentrates on those birds that live and breed within the Arctic Circle. Aimed at readership on both sides of the Atlantic, Malcolm Ogilvie begins his book with a chapter describing the nature and extent of the Arctic followed by another on the adaptations by birds to Arctic conditions. Four chapters follow on waterfowl, sea, shore and land birds found in the Arctic, and the book finishes with a chapter on the need for conservation in the Arctic. Generally the text maintains a high level of interest but I

Above illustration: *The Archangel Gabriel, part of a miniature from Syria or Egypt (14th century, British Museum, London). Taken from Islam and the Arab World, reviewed on page 596.*

wondered if giving each bird species found in the Arctic was the best way of dealing with this group of birds. Some accounts are interesting, particularly those on waterfowl, which are the author's speciality; and on waders, such as knot, on which much research has recently been carried out. But some other sections are mere repetitions of textbook material. Chapters describing and comparing the characteristics of individuals of the group in the Arctic might have been far more readable and have provided an opportunity for more discussion. Maps and photographs are useful additions to the text as is the up-to-date bibliography. The book is well produced—recommended for Arctic enthusiasts.

Tony Soper's book *Everyday Birds* (David and Charles: Newton Abbot and London, £2.95)—a very popular account of the lives of nine very common birds—will please children and the less discriminating adult bird lover. Much of the text is very simple, factual and moves at a great pace, which is possibly why he makes the occasional careless statement such as "birds are controllers of insects" and "birds have a great sense of adventure". This book will be lapped up by a large market who will also enjoy Robert Gillmor's excellent drawings. □

Peter Conder has recently retired as Director of the UK Royal Society for the Protection of Birds.

Spirit of Nature needs rekindling

OVER the past ten years, the study of animal social behaviour has undergone a dramatic change. Gone are the days when animals were held to do things 'for the good of the species' or for the benefit of their group if it meant that they themselves suffered in the process. Altruism—in the sense of behaviours which benefit the reproductive success of other unrelated individuals at the expense of an animal or its relatives—is contrary to the Darwinian theory of natural selection. Selfishness and exploitation are the rule. So harsh a master is natural selection that it is not even the individual that benefits in the long run, but his genes. The individual animal has a finite lifetime, but his genes, in the bodies of his offspring, and in the offspring of his brothers and sisters or other relatives live on. Hence any genetic propensity to benefit relatives will be increased because the relatives will also be likely to have the same propensity. This idea of 'kin selection' has been fully developed by W. D. Hamilton.

In view of this shift of thought ('revolution' is too strong a word: it is more a realisation of what was implied all along by the theory of natural selection), it is disappointing to find that so many recent popular books still perpetuate the fallacy that the species is the unit of selection and seem unaware of recent developments in the field of evolutionary biology. This is particularly apparent in *The Mating Game* (Elsevier-Phaidon: Oxford, £4.50) by Robert Burton, a lavishly illustrated account of animal courtship and reproduction. The photography is most impressive, but the text is full of such statements as (p10) "the individual is unimportant and survives solely for the benefit of the whole", and (p8) "the

function of sex is thus to ensure that animal populations are composed of a variety of individuals". The function of sex is one of the most hotly debated issues among evolutionists precisely because 'good of the population' arguments have been found to be inadequate. It may certainly be advantageous for the population as a whole to be variable, but the problem is that sexual reproduction involves producing individuals that contain only half the genes of each parent, an apparently enormous cost genetically, which asexually reproducing individuals would not have. The work of R. L. Trivers has made us take a new look at animal courtship and pair bonding. Animal family life is seen as a world of exploitation of one sex by the other, of cheating and of strategies involving cooperation only when this is selfishly the best thing to do. There is no mention of this aspect in the book, nor any of the genetic relationships between the members of a social insect colony, which is crucial to an understanding of their curious reproductive system.

Something of the same fallacy is apparent in John Napier's book *Monkeys without Tails* (BBC: London, £5.25), which on p33 states that "the whole life style of animals is a preparation for ensuring the continuity of their species" and that "animals that do not reproduce are biologically irrelevant". On the contrary, kin selection theory has shown that non-reproducing individuals may be genetically very important. This book, although a very interesting account of primate biology does not quite live up to its subtitle—*A Giraffe's-eye View of the Evolution and Life History of Man and the Chimpanzee*. The idea is an appealing one—to look down on man from a great height as a rather ordinary primate—but the book is nevertheless rather noticeably anthropocentric. Man is placed at the top of the primate

"staircase", with the "great divide" separating us from our primate relatives. There is a lot of useful information in this book, however, and it is very readable.

The Secret Life of Animals (Weidenfeld and Nicolson: London, £8.95) by Lorus and Margery Milne and Franklin Russell also insists that (p112) "whatever the behaviour is that best suits the survival of the species, occurs", but is redeemed by some really superb photography. The book really does succeed in conveying a sense of awe and wonder about animal life, which *The Amazing World of Animals* (Thomas Nelson: London, £4.50) edited and with a foreword by Sir Peter Scott also sets out to do. This book contains some good photographs, and chapters on different animal groups by various authors.

The Classification of Animals (White Lion: London, New York, Sidney and Toronto, £3.75) by Richard Freeman is a most useful illustrated summary of the classification of living animals, with examples, a clear indication of the numbers of species in each group covered, and a brief but informative description. It is concise, but at the same time satisfyingly comprehensive—the basic taxonomic information needed by a zoologist in a few pages.

The Language of Smell (Routledge and Kegan Paul: London and Boston, Massachusetts, £3.95), also by Robert Burton, is a summary of how animals smell and the uses to which they put their sense of smell, at a fairly elementary level. The recent evidence that shows that von Frisch has been right all along in claiming that the dances of honeybees transmit directional and distance information, is apparently unknown to the author. One is therefore, mistakenly given the impression that von Frisch is now generally considered to have been wrong and that bees rely on smell alone.

It is a relief to turn to *Bird Life: An Introduction to the World of Birds* (Elsevier-Phaidon: Oxford, £5.95), with a text by Christopher Perrins. Here at least the basic evolutionary framework is sound and the current thought on social behaviour, mating, and so on, is simply and well explained. The book covers in a readable way various aspects of bird life, such as feeding, social behaviour, migration and so on. It is clear, non-technical and has numerous coloured drawings by Ad Cameron, which although expert and lifelike, might perhaps be thought a little monotonous as the sole source of illustration.

Marian Dawkins

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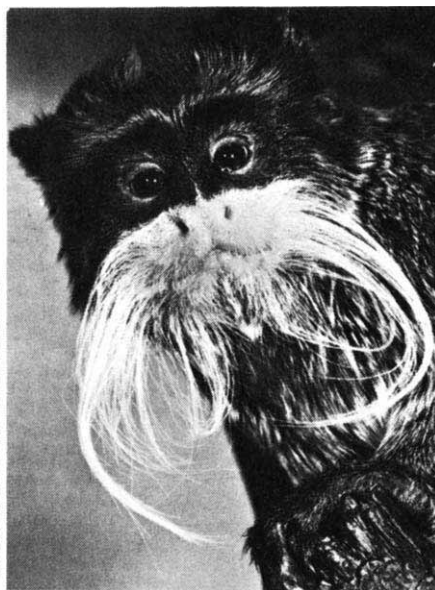
Capturing the secrets of Nature

NATURAL HISTORY must still be the subject most likely to kindle an interest in science in the tinder-dry mind of a young child. The problem is how to tend the flame so that the interest becomes a passion. When I was a child the process included Nature walks, listening to BBC radio's *Nature Parliament* and *How Things Began* and, of course, books. Now, streets ahead of anything else, there is film.

Film allows us and our children, at home and at school, to view every intimate detail of the erstwhile secrets of Nature. No longer do we wonder what goes on in the darkness of a woodpecker's nest nor contemplate the riddle of the landing of a fly on the ceiling. Given a steady diet of fantastic film, can the jading palate of today's child still be tempted by a book on Natural History? To judge by the masses of such books on the market the answer must be yes, or at least the adults who buy the books must believe so. For a book to be successful, however, it must surely attempt to match rather than ignore the dramatic qualities that the moving film has brought us. Only one of three series currently available really does that.

The success of the *A Closer Look At . . .* series (Hamish Hamilton: London, £1.50 each) rests heavily on the illustrations and the consistently good, sometimes inspired, layout. There are no photographs in these books. Instead the pages are generously supplied with full colour drawings which, at their best (for example, in . . . *Elephants* and . . . *Apes*) are vivid double-page spreads capturing a group of animals in mid-action. Each double page also has a few hundred words of text addressed to a particular topic, which vary from book to book but which generally start with evolution or geography and close with the prospects for . . . Information in the main part of the book is, however, centred either on species or behaviour. With such a limited amount of text the choice of topics or facts has inevitably been slightly arbitrary but a certain balance has nevertheless been maintained in each volume.

The publishers of the series make no claim as to the age group for which it is suited. The books certainly succeed as picture books for the six year old—particularly the more gory offerings, such as the death by leopard of an Australopithecine boy (. . . *Prehistoric Mammals*) or the spider-killing wasp (. . . *Bees and Wasps*) and I am sure that the text would carry the series up to the start of the teens. There are occasional oddities: the concept of



Emperor tamarin (marmoset monkey)

Taken from *How Mammals Live*

nidifigous and ridiculous birds may be worth a page in the . . . *Birds* book but surely one could do without the terms themselves. And failings: the quality of the illustrations in . . . *Arctic Lands*. But overall these are excellent and reasonably priced books.

The second series, to which *How Insects Live* (Elsevier-Phaidon: Oxford, £4.50) is the latest addition, is clearly, if not explicitly, aimed at the teenager. But it would have to be an already committed one who actually benefited from the books. That, to my mind, is the failing of the series. In general design the volumes resemble a 'coffee-table' production both in size and in the abundance of handsome colour photographs. But the design belies the text which is by turns dull, didactic or of the "just-fancy-that" variety. Where

exactly did the editor expect to find readers whose attention would be captured by Haeckel's dictum that "ontogeny repeats phylogeny" (*How Fishes Live*, £3.95) or by the correlation between the breeding behaviour of ring doves and the weights of their oviduct and crop contents (*How Birds Live*, £3.95)? Perhaps the bookish student, aged 15 or over, will appreciate these volumes; otherwise, buy them only if you cannot resist the photographs.

The *Nature's Way* series (G. Whizzard/André Deutsch: London, £1.75 each) is to my mind an even greater failure. The origin of this series is the Oxford Scientific Film Unit which is rightly renowned for its Natural History films. These books (*Bees and Honey*, *Life and Times of the Stickleback*, *Butterfly Cycle*) are clearly spin-off material. Each consists of about five pages of simple text followed by about twenty full page coloured photographs. The text is unexceptional, and the photographs are generally fine, but I was dissatisfied, perhaps through frustration, at the inevitable failure to translate action into stills photography. Once through these books was enough for me, and would, I suspect be enough for children. Not enough, however, to be able to recommend them as worth buying.

I have surprised myself by coming down heavily in favour of the series of books that relies on artistic rather than photographic illustrations. If you want the 'real' thing I can only say stick to television or, far better, head for the country.

Peter Newmark

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Nature on the seashore

ALTHOUGH books about the seashore might seem more relevant as a preparation for summer holidays, they may nevertheless make acceptable Christmas presents for scientists specialising in other subjects, laymen or the generation still at school or university. Two books have appeared in the shops during 1976 and it is interesting to compare them with some other books written over the last 27 years which are still available.

Taking the new books first, we have *The Hamlyn Guide to the Seashore and Shallow Seas of Britain and Europe* by A. C. Campbell (Hamlyn: London and New York, £2.25). This book is bound in durable paper that might withstand rain or seawater to a limited extent during shore collecting. The illustrations, by James Campbell, all in colour, are well above the average for this type of book. After a very brief

introduction on types of seashore, tides and collecting, the non-biologist is thrown in at the deep end with two pages of "key" which cover the entire plant and animal kingdom. The reader must then find his or her way to the right phylum where a brief description of the phylum is followed by a list of species divided into classes and families. It is essential for the reader to find the plant or animal by its picture. Once this has been achieved the genus, species, authority, popular name, size, brief description, habitat and distribution are given. At the end of the book there is a glossary, bibliography and index. For identifying shore life, this book may well be the best available on the market and at its modest price is strongly recommended for the amateur shore collector.

The second new book, *A Field Guide to the Mediterranean Seashore* by W. Luther and K. Fiedler, translated and edited by P. J. Miller (Collins: London, £4.95) is a hardback with a cover of what seems to be

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at the University Press, 22 George Square
Edinburgh

Circle No. 13 on Reader Enquiry Form.

waterproof material. After a somewhat longer introduction on habitats, each phylum is described in turn and its representatives given by class, order and family. Although each species is briefly described with Latin and popular name, size and habitat, the illustrations are much poorer than the Hamlyn book: many are only black-and-white, and those in colour are given against a coloured background that seems to provide an unnecessary and sometimes confusing contrast. The book ends with a section on collecting, marine aquaria, a bibliography and index. There is no glossary or general introductory key so the non-biologist, although he might find the book a useful companion on a Mediterranean holiday, may have difficulty in tracking down a specimen.

Collins' Pocket Guide to the Seashore by J. H. Barrett and C. M. Yonge (Collins: London, £3.95) is still being reprinted as the 1958 edition in hardback. Covering both plants and animals, it is divided into phyla and the main subgroups (down to species) to be identified by a proper key. Having at last arrived at the species, the Latin and popular names are given, with a fairly full description of anatomy, habitat and distribution. This is a more difficult book for the amateur (or

professional) to use; not all species are illustrated; and the illustrations (partly black-and-white, and partly coloured plates of good quality) are placed together near the centre of the book and not with the species description. There is a bibliography, index and a useful introduction. According to the introduction the book is aimed at amateur naturalists, who will probably find more species described than in the Hamlyn book but will certainly require more patience in locating their specimens from 'first principles'.

In very different style is *The Seas* by F. S. Russell and C. M. Yonge (Warne: London and New York, £6.95). First published in 1928 and reviewed in its revised form by Sir Cyril Lucas (*Nature*, 258, 36, 1975) we find a book divided into 17 well-illustrated chapters. This is not a book for identification or reference; it is a collection of essays in non-technical language on various aspects of marine biology. The book is readable, and would provide the amateur with a good general picture of all aspects of the sea and sixth-formers or first-year university students with worthwhile background information.

Equally well-produced is *The Sea Shore* by C. M. Yonge (Collins: London, £6.50 hardback, £0.95 paper-covers). This is one of the celebrated New Naturalist Series originally published in 1949, reprinted last in 1971

and still available. It is at a similar technical level to *The Seas* but the 19 chapters are restricted to the shore areas, with a more detailed description of subjects like Life in Rock Pools, Zonation on Rocky Shores, and Life on Sandy Shores, Mud or in Estuaries. The book is beautifully illustrated and highly readable and will take a rightful place with others of the New Naturalist Series on any bookshelf.

Finally, *Fishes of the Sea* by J. N. and G. Lythgoe (Blandford: London, £3.90 hardback). Although described as a coloured photographic guide to the fishes of the British Isles, Northern Europe and the Mediterranean, it consists also of an introduction to fish anatomy as well as descriptions and line drawings of each species. There are many coloured and black-and-white underwater photographs placed together at the centre of the book which might be used by SCUBA divers for identifying fish in their natural surroundings. The book is thus of restricted value to the shore collector and non-diver, but would be a worthwhile addition to the luggage of amateur divers holidaying next summer either in the UK or on the continent.

J. H. S. Blaxter

Dr Blaxter is Senior Principal Scientific Officer at the Dunstaffnage Marine Research Laboratory of the Scottish Marine Biological Association at Oban, Argyll, Scotland.

Enthusiasm for shell collecting

The Shell Collector's Guide: An Introduction to the World of Shells. By S. P. Dance. Pp. 192. (David and Charles: Newton Abbot and London, August 1976). £4.95.

SHELL COLLECTING or conchology is among the oldest known intellectual leisure activities. The fascination of "shells that speak seven seas" (one of the many apposite quotations from unlikely sources—this one is from Dylan Thomas) is evidenced by shell jewellery from archaeological sites about thirty thousand years old. More recently, excavations at Pompeii have unearthed shell collections, evidence of the interest in conchology that this book will doubtless help to perpetuate. Peter Dance has written an enthusiastic, honest, technically expert and enjoyable book about a semi-scientific hobby which can bring great contentment (and occasional heady excitement) to young and old alike.

Many naturalists have tried to put into words something of the fascination of conchology. As a hobby, it is all

things to all men. It appeals to the outdoor person who wants to punctuate his rambles by rustic searches; to the SCUBA diver and his less adventurous wading or dredging comrades; to the young who marvel at the exquisite geometry of shells; to the old who take satisfaction from their patient systematic accumulations over the decades and from postal exchanges with enthusiasts all over the world; to those of an artistic persuasion who love the beauty of shells; to scientists who yearn to make order from chaos and enjoy arranging, naming and cataloguing their collections. In short, first and foremost, conchology is FUN. It can, and occasionally does, lead to advances in the scientific study of the Mollusca, and can bring rich intellectual rewards to those who embrace it. It can also be physically pleasant to handle shells, although not everyone would agree with Mr Dance that touching cowrie shells (for example) provides a "decidedly sensual tactile satisfaction".

This book will tell you everything you need to know to begin searching for, cataloguing, preserving and displaying a shell collection. It tells you about shell clubs, shell shows, valuing,

exchanging and buying shells. Fascinating sections of the book deal with shell frauds, and with the commercial exploitation of the rarest shells. Did you know, for example, that prices of certain shells at auctions have now topped the \$4,000 mark?

One of the best features of the book is the high quality of the illustrations, with a splendid series of photographs by Ian Cameron and well chosen and beautifully executed drawings by Annabel Milne. The standard of production of this book is uniformly excellent and it is pleasant to note that it was printed and assembled entirely in Britain.

Why then is my brow furrowed? Simply because I share with many other professional zoologists a frightful vision of a world in which all the molluscs are on display in skilfully designed cabinets, and the seas, lakes, marshes, streams and woods are malacologically empty. If anyone doubts that this is more than an irrational nightmare, he should ask himself how many butterflies he has seen in Britain during recent years; have lepidopterists any share of the blame for their dearth?

The author offers no balm for my anxieties about the possible depredatory effects of indiscriminate shell collecting. He advocates searching for living molluscs (so that they may be killed for their shells) rather than taking empty shells from the beach deposits, and describes in detail various unpleasant methods of torturing the animals out of their shells after capture. He describes one method which is so hideous that he says he never himself has practised it (why then lend it currency?).

About wholesale commercial conchology (reef raping, as an Australian described it) he lamely says he does not propose to take sides or to argue the rights and wrongs. About the legitimate and calmly voiced fears of naturalists about the future of the mollusc world, wanting to keep the word 'conservation' before the shell collector, Mr Dance says only this: "For some mysterious reason, the scientific world appears to think that it has almost a divine right to Nature's products". This is unfair. Speaking personally, I do not want to conserve molluscs simply so that I and others in my laboratory can catch and experiment on them; I want to conserve them so that my grandchildren (and Mr Dance's) can see and admire them. I hate to feel that I may be living in the last generation of mollusc enthusiasts.

T. E. Thompson

T. E. Thompson is a Reader in Zoology at the University of Bristol, and President of the Malacological Society of London, UK.

Artistic impetus for scientific observation

Bright Wings of Summer: Watching Butterflies. By D. G. Measures. Pp. 160. (Cassell: London, October 1976.) £5.

THIS book is the work of an artist. David Measures goes out to his disused railway line in Nottinghamshire and draws and paints, describing what he sees in the butterfly world at various times of the year and in different weather conditions. He reproduces his notes and his delightful sketches, full of movement and swift life, and in the past twelve years he has collected a great deal of first-hand information on topics such as scents, courtship, mating, egg-laying and territorial boundaries in a unique way which is not to be found in the standard books.

The object is to set out the life histories and behaviour of butterflies in a way which will stimulate readers to become interested in Natural History and to make and record their own accurate observations. The unfinished paintings and the roughly written notes show how in practice conclusions are arrived at.

The book is divided into three parts. In "The Beginnings" he describes his own life history. He started with a general love of nature and then, because of his preoccupation with colour, concentrated on butterflies. "There is nothing so brilliant, or as changing and shifting as the iridescence and the pigmented colour of the butterfly's wing".

Part 2 gives the results of his observations, and lastly, "You and

Butterflies" gives practical advice as to how to follow in his footsteps.

The lesson for the pure scientist is that each insect is an individual and that, when one classifies, something is lost—although for a physician-reviewer accustomed to looking after patients this does not come as a surprise.

On the other hand, art must not be allowed to get away with it completely, and with more scientific knowledge the author's observations could have been more productively channelled. What precisely happens to our red admirals in the autumn? Burnet moths are resistant to cyanide, and cinnabar caterpillars feed on the poisonous ragwort—what are the predators of these insects? Do the black and white forms of the peppered moth actually settle on appropriate backgrounds? Similarly, where in nature do caterpillars with brown and green chrysalids actually pupate?—the colour and texture of the site are of great interest. The extent of bat predation at dusk also needs investigating.

Other criticisms are that there should be more emphasis on the difference between camouflage and warning coloration (p 72) and surely the plate opposite page 100 is not the small heath. In general, however, the photographs are superb.

The book can be strongly recommended for nature lovers with artistic inclinations, but there is no reason why imagination and science should not be brought closer. E. B. Ford's *Butterflies* (which is recommended for further reading) exemplifies what I mean.

Cyril A. Clarke

Sir Cyril Clarke is a general physician with an interest in genetics which stemmed from breeding butterflies. He is President of the Royal College of Physicians.

Jumbo the elephant

Jumbo. By W. P. Jolly. Pp. 173. (Constable: London, September 1976.) £3.95.

BORN into a matriarchal society in the wild bush of Africa, captured and transported to the Jardin des Plantes in Paris, the 4-year-old elephant was then exchanged for a rhinoceros, a piece of trading which almost certainly saved him from a stewy end in the siege of Paris of 1871. A more tranquil period followed with little to do to earn an almost limitless supply of buns other than carry wide-eyed children on rides round London Zoo.

Jumbo, for this was his name, thrived in London and grew apace. Jumbo was no more than a name of African origin but one which was to come into everyday use and be synony-

mous with largeness. The period of adolescence ended in pain of two kinds, the physical pain from abscesses which formed following the breaking of both tusks and the pain and puzzlement of puberty which seems to afflict the male elephant in the constraining environment of captivity.

The physical pain was assuaged by the surgeon's lance but the latter required very pragmatic action, if the embarrassment of an Elephant amok in the Zoo was to be avoided, and resulted in Jumbo's sale to Mr Barnum, the great American showman. The bill of sale was easy; the delivery of a reluctant elephant was, however, fraught with difficulties, difficulties that resulted in enormous publicity which promoted the forthcoming arrival to the American people and increased the anguish of the British public at the loss of their animal hero.

How little times have changed. Good organisation and one suspects the right kind of word from the animal's keeper, who could not but fail to profit from the increasing fame of his charge, and despite attempts for an injunction against the sale, Jumbo was loaded on the *Assyrian Monarch* and after an uneventful journey arrived in New York in April 1882. There then followed the razmataz of the American circus life which continued until Jumbo's death following a collision which Special Freight Train number 151 just over 3 years after his arrival in the New World.

The author draws from many sources to relate the tale of Jumbo's life and times. He links together the extracts from that period in a way which at times I found rather tedious, phrases like "yester year's politicians prize from China" to describe Mr Ted Heath's contribution of Pandas to London Zoo is but one example. But this could not spoil a fascinating story of a most unusual animal. The book is a must for all Jumbophiles.

R. J. Wheeler

Mr Wheeler is Director of the Royal Zoological Society of Scotland.

The Bethnal Green Museum of Childhood, London, UK, has mounted a special exhibition (November 25, 1976-January 23, 1977) of material related to the story of Jumbo. The exhibition also includes a wide variety of examples of the elephant in art, and contains toy elephants and a selection of photographs and drawings from children's books.



Jumbo with admirers at London Zoo

Photo: Zoological Society of London

Snapshots in the field

Field Photography: Beginning and Advanced Techniques. By Alfred A. Blaker. Pp. xxi+451. (Freeman: San Francisco and Reading, 1976.) \$19.95; £12.60.

THE camera is used by scientists to observe, collect and record data and to help communicate the results to fellow workers. Most government scientists can call on the services of professional photographers who produce photographs that are technically superb. But even these fortunate scientists, as well as ones without such facilities available, need to take photographs themselves as the opportunity presents itself, and this is especially true for those whose work takes them into the field.

The scientist who takes his own photographs has the advantage that he knows exactly what he wishes to portray. The result is, however, too often spoilt and rendered unfit for publication by lack of the proper equipment for the job, or the lack of rudimentary technical knowledge. One of the primary aims of *Field Photography* is to provide sufficient information for the scientist to overcome these difficulties. All too often articles and books on modern photography are

either technically too complex for this purpose, or consist merely of a series of photographs joined together with the minimum of words or explanation.

Field Photography is noteworthy for its balance of text and illustrations. It can be readily understood by the beginner and yet contains much that is of interest and help to the photographer with some knowledge or one who has experience in some other branch of photography.

The steps needed to perform a certain operation are always given clearly and at length. Sufficient information is always conveyed for the reader to understand the reason for the processes involved, but the theory underlying the operation is usually only given at length when needed to enhance the practical performance. And the author does not dwell unnecessarily on the chemical or physical aspects of photography.

The book is divided into three major parts. In the first, the reader is skillfully piloted through the maze of photographic equipment and materials that are available, including the basic types of cameras, lenses, extension tubes, bellows, filters, light meters, flash meters, tripods, film and printing paper. The particular advantages and disadvantages of various items of equipment are clearly discussed: even veteran photographers should occasionally pause to reflect

whether the system they have adopted or evolved is really the most suitable for the job at hand, or whether it merely reflects ingrained habit.

In the second part of the book general photographic techniques are reviewed, emphasis being placed on: picture composition; exposure, including the use of light meters and electronic flash techniques; the purpose and use of filters, especially as they relate to scientific recording in the field and their use in infrared and ultraviolet photography; and darkroom procedures.

The third part is specially concerned with field photography techniques and is in many ways the most valuable. There can be few photographers who could not learn something from it. The subjects discussed include: climatic problems and protection of equipment; high resolution techniques, including camera handling, film processing and printing; macro-photography, including the use of supplementary lenses, teleconverters, extension tubes, and lens reversal; use of telephoto and wide angle lenses; techniques of stereophotography; and use of fill-in flash. The few pages in this section that are devoted to the correct disposal of photographic waste material and the avoidance of damage to the environment would seem unnecessary to the scientist who habitually works in the field.

The book is well produced and the clarity of presentation is enhanced by some excellent and purposeful black-and-white and colour illustrations, and many line drawings, figures and tables. Included with it is a booklet in which many of the mathematical formulae and tables used to calculate lens apertures and shutter speeds are reproduced, so the photographer can have readily available information with him in the field.

Field Photography is good value, especially if it succeeds in its object of instructing the scientist who buys it to take photographs outdoors efficiently and economically.

John B. Free

John Free is an entomologist at Rothamsted Experimental Station, Harpenden, UK, and a professional natural history photographer.

Volcanoes

Volcanoes. By Peter Francis. Pp. 368. (Penguin: Harmondsworth, Middlesex, July 1976.) £1.50. *Volcanoes*. By A. and L. Rittmann. Pp. 128. (Orbis: London, 1976.) £4.95.

"THERE are many books on the market called *Volcanoes* . . . so why write yet another?". Thus begins Peter Francis in his addition to the *Pelican Original* series. And he has his own answers to the problem. Volcanology, he explains, is a fast-moving discipline, both conceptually and spatially. Studies that once encompassed little more than explicit phenomena on the surface of the globe now extend from the Earth's core to the far side of Mercury. Then there are the hidden implications of volcanoes. On the surface a volcano may be just a volcano, but an eruption has far reaching ramifications often ignored by other authors. And finally, most treatises on volcanoes are textbooks.

Peter Francis wanted to write one free of "academic pretensions" for a less than specialist readership, and his attempt at meeting that objective is an unqualified success. Here is a gem of a book; from the outset his knowledgeable fascination with volcanoes and all they represent shines through. Gifted with a lucid and breezy style he carries his narrative across every major facet of volcanology, penetrating surfaces just deeply enough to provide sufficient foothold for the next conceptual step. Nobody will get lost along the way, and few will want to abandon the journey.

The book proceeds logically, condensing a mass of detail as it does so. It starts clearly with a description of

volcanic distribution—the Mid-Atlantic Ridge, the Pacific Ring of Fire, and so on. This, of course, is merely plate tectonics from another angle, but it provides the author with the opportunity of introducing the fundamental geophysics of volcanology and all that that entails—from basic mantle chemistry and convection cells, through oceanic magnetic anomalies, to the Low Velocity Zone.

The author proceeds to paint a vivid picture of what volcanoes are and what they do. The scene is set with a straightforward descriptive chapter on three diverse "classic eruptions"—Vesuvius in AD 79, Krakatoa in 1883, and Mont Pelée in 1902—which, with the graphic inclusion of asides on the plight of unfortunate victims, provides a clear idea of the varied ways in which volcanic action can manifest itself.

But it is the following chapter which perhaps best highlights the author's skill as a scientific narrator. Dealing with the classification of eruptions, again everything is there—central and fissure eruptions, basaltic flood eruptions, Vulcanian and Strombolian eruptions, Hawaiian eruptions, and so on. The obvious pitfalls are confusion and complexity; but Francis does not trip up, making his central point clearly and concisely.

Lava types (a few words here on mineral chemistry and petrology), pyroclastic falls and flows, volcanic morphology and volcanic evolution are then considered, and by the end of chapter 7 the story could have been complete on many counts. But Francis is scarcely more than half way through. As he says bluntly: "a volcanic eruption does not take place in an environmental vacuum". So we get *hlaups* and *jökullhlaups*, volcanic pollution, *tsunamis*, climatic effects, volcanoes and the origin of life, and even vol-

canoes as money spinners—ores, geothermal power, and the like. A chapter on volcanic prediction and mitigation (La Soufrière is topical), a journey out to Mercury (by way of the Moon and its history, and Mars), and at last the text reaches its breathless end.

Peter Francis' *Volcanoes*—361 pages crammed with information—is thoroughly comprehensive and crystal clear. Written to inform non-specialists, it does so with panache.

By comparison, *Volcanoes* by Alfred and Loredana Rittman is a strange offering, the more so in view of the senior author's standing in his field. As the dust cover proclaims, he is indeed a "world authority"—an ex-President of the International Association of Volcanology, and founder of the International Institute for Research into Volcanology.

With that in mind his latest contribution emerges as a sadly disappointing work. It really belongs to that peculiar breed known as the 'coffee-table' book. Without doubt, the 120-odd full-colour pictures are breathtaking—of the type that beg to be looked at time after time. But the real difference between this book and Francis' (and given the similarity of textual approach the comparison is valid), is that whereas Francis writes to invest his photographs with life, the Rittmanns' present a relatively abtuse and somewhat superfluous text that is far outdazzled by the pictures. So if you have a coffee table and a penchant for Kodachrome catastrophe, the Rittmanns' *Volcanoes* is the book for you; if not, spend your money on the other option.

Allan Piper

Allan Piper is Assistant Editor of The Oilman.

Astronomy for the amateur

STARS and planets, galaxies and space are topics of tremendous interest to many non-scientific members of the general public. In fact astronomy is second only to natural history in the layman's top ten of scientific subjects, and this is amply reflected in the plethora of popular astronomy books published every year. These books are difficult to classify but the seven under review fall into four basic groups, the encyclopaedic all-you-want-to-know about astronomy from A to Z; the picturesque, where a large collection of glossy photographs are interspersed

by brief descriptive texts; the armchair tomes for the fireside astronomer; and the 'get-up-and-go' books which encourage you to actually go outside and gaze, hopefully with wonder, at the heavens.

First let us consider the two encyclopaedias. These ABCs of astronomy are ideal for people who want to look up simple astronomical facts and who want quick answers to questions without having to study a lengthy chapter in a book. A. Weigert and H. Zimmermann's *Concise Encyclopaedia of Astronomy* (Adam Hilger: Bristol, £9) is a revised and expanded second edition of the original *ABC der Astronomie* first published in 1967. There are over 500 pages of facts, many illustrations, sixteen plates and a fine collection of star maps going down to

fifth magnitude. This book is for the advanced amateur and is also an excellent reference book for first-year university students. Simple mathematical relationships between quantities are effectively used and the tabulation of stellar and planetary data is first rate.

The Illustrated Encyclopaedia of Astronomy and Space (Macmillan: London, £7.95), edited by Ian Ridpath, contains contributions from twelve or more people from diverse astronomical backgrounds. This encyclopaedia tries to bring together astronomy and space science, and amongst the 1,000 entries are some very informative tables giving details of such space programs as Apollo, Surveyor, Soyuz, Discoverer, Salyut and Skylab. The book is equally divided between space and astronomy—for example, the planet Saturn is allocated as many column inches as the Saturn rocket. In fact I think the book goes too far into space—Frank Borman the Apollo 8 mission commander is allocated eleven lines, and the fundamental astronomical concept of black-body radiation is afforded scant reference in the section on spectroscopy. It is well illustrated but is much more simplistic than the work by Weigert and Zimmermann. Ridpath's book reads more like an astronomical *Guinness Book of Records* and seems to be aimed at people in their early teens.

The Southern Universe (Macmillan: London, Melbourne and Sidney, £4.95) by Lennard Bickel is so lavishly illustrated that it can almost take its place with those other 'coffee-table' books designed just to be flipped through to look at the pictures. This is, however, a bit unfair as the text is worth reading especially if you can overcome your dislike of journalistic flamboyance:

"The Clouds of Magellan are portals to outer space. Their location—50,000 parsecs above the South Pole—is our threshold to the greater universe, an arena so vast it is better described as—the cosmos. This is the great empyrean of the heavens, the region of supreme creation where scientific astronomy is joined by cosmology and modern physics in the search for understanding of the true Beginning."

Yeuk! There again it might be just the thing for the kiddies. Interestingly the book is written by an Australian, with the cooperation of many Australian astronomers and with a strong emphasis on the southern sky, a refreshing change from the usual bias towards the northern hemisphere.

Isaac Asimov writes for the armchair astronomer, and his book, *Eyes on the Universe: A History of the Telescope* (Andre Deutsch: London, £4.95), sets out to tell two stories. First, a fascinating account of the technological

achievement that developed the telescope from the first Heath-Robinson contrivances of the pioneer astronomers to the giant optical and radio telescopes of today. And second a narrative of mankind's supreme intellectual adventure as he reaches out and tries to comprehend the nature, scope and origin of the Universe. The book succeeds admirably and tells its rather complex tale, without the use of mathematics, in a clear and succinct way. A perfect example of good scientific popularisation.

Astronomy for the Amateur (Macmillan: London, £2.95) is a short, well illustrated introduction to astronomy. John Gribbin has written for those people "who have noticed the fascinating lights in the sky and would like to know a little more about them". It is a guide for people embarking on astronomy as a hobby, tries hard to keep alight the fascination and wonder of this exciting science, and succeeds very well.

Moving outside we come to James Muirden's *Astronomy with Binoculars* (Faber and Faber: London, £4.95 cloth, £2.75 papercover), a new and revised edition of the book first published in 1963. Astronomy is an intimidating science, many newcomers imagining that astronomical research can only be carried out in big observatories. Muirden tells us all to get outside on the frosty winter nights and train our binoculars on the sky. There are chapters on the observation of the Sun (needless to say *not* with binoculars), Moon, planets, comets, meteors aurorae and stars. In each case the author describes in detail what we should be looking for and prefaces these descriptions by a brief introduc-

tion to the physical conditions prevailing on the object. This book conveys all the excitement and challenge of astronomy, and the owner of a pair of binoculars will find the book worth every penny of its cost.

Finally, I come to *Stars and Space 77* (Independent Newspapers: London, £1) a ninety-six page, magazine-style paperback, which firstly describes the night sky for every month of the coming year—giving well drawn and very clear star maps plus details of planetary positions and lists of interesting stars to look out for. The second half is given over to reviews of the major advances and discoveries during the past year. These three- to four-page articles cover such subjects as exploding black holes, X-ray satellites, other planetary systems, the solar influence on the Earth's weather, comet West, and Viking. The author list reads like a Who's Who in Astronomy, and Ian Ridpath, again editor, must be congratulated on an excellent publication—fantastic value for money at only £1. Heaven help the sales of Patrick Moore's *Yearbook of Astronomy*.

Looking at all these books the question to ask is: "Which, assuming you are a young astronomer or a recent addition to the realms of amateur astronomers, would you like to find in your stocking on Christmas Day". The choice is enormous, but for me the answer is simple. James Muirden's *Astronomy with Binoculars*, wins hands down, with Ridpath's *Stars and Space 77* also making a superb and cheap stocking filler.

David W. Hughes

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Ecology of human skin

The Life That Lives on Man. By Michael Andrews. Pp. 183. (Faber and Faber: London, 1976.) £4.95.

TELEVISION has brought many aspects of science vividly to huge audiences, and the programme *The Life That Lives on Man* did this most successfully. It gave an accurate picture of the human skin, and had shown—with shots of dust pouring out of our underclothes—how we shed some ten thousand million tiny skin scales a day, losing in a year a weight of over a pound from our tissues. It had also shown photomicrographs of the bacteria and the various arthropods which live on and in the skin, and described the diseases some parasites may cause. There were accounts of the research

on these parasites, and reconstructions of some of the unusual experiments (including those in which man was the victim) which contributed to this research. It was obvious to the viewer that an immense amount of work had gone into preparing the script, and in taking and editing all the photographic material. It seemed a pity that so much effort should produce so little permanent result, for even the most attentive viewer retained only a fraction of the information which illuminated his screen when the programme was presented on television.

It was therefore an excellent idea when Michael Andrews decided to write "the book of the film". In it he has been able to include much that his research revealed which could not be shown on television because of pressure on time or because not all aspects of the subject are equally photogenic. Mr Andrews has produced a fascinating

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Latching lice, by Bartolomeo Finelli (Kome, 1781-1835)

book, scientifically accurate and yet free from irritating jargon. It should appeal to the non-scientist, and yet has something to teach the specialist, including the dermatologist and the parasitologist.

Although the text is enlivened with historical anecdotes and literary allusions, with poems about the parasites and epigrams at the expense of the parasitised, the book is fundamentally an exercise in ecology—the ecology of the human skin. We learn that the millions of bacteria which inhabit it are normally in balance, although many are potentially the cause of serious disease. When man does not interfere by washing too frequently, or applying potent chemicals to neutralise natural body odours (not 'BO', which is generally a pathological symptom) and to prevent natural sweating, the various micro-organisms can usually co-exist in mutual harmony. Dangerous pathogens are somehow prevented from increasing to damaging numbers. It is clear that many bacteria and other micro-organisms are beneficial and not harmful to their hosts, a situation recently described in Bernard Dixon's book *Invisible Allies*. The skin is seen to behave much like other, larger, ecosystems.

Stability may, however, be upset in all ecosystems, and the skin is no exception. Dangerous bacteria do sometimes get out of control, perhaps because man, with his unguents and cosmetics, has upset the balance. It must also be admitted that it is difficult

to determine in what ways most arthropod parasites contribute to the well-being of the host which supports them. Lice suck our blood, irritate our skin so that we scratch and admit pathogenic bacteria to make the wounds fester; and the insects also infect us with dangerous rickettsiae which cause epidemic typhus. Fleas bite and inoculate us with the plague bacillus—the cause of the deadly 'Black Death' of the Middle Ages. These infestations

Coffee-table medicine

Triumphs of Medicine. By H. Keen and J. Jarrett. Pp.193. (Paul Elek: London and New Hampshire, 1976). £12.50.

LAVISHLY illustrated books on art, history, architecture and travel have proved so successful that from time to time a publisher attempts to apply the same technique to a scientific theme. The size (30×23 cm) of this Paul Elek production puts it firmly into the 'coffee-table' class, but it is far from a typical example—except in its high price.

Professor Harry Keen and Dr John Jarrett set out to describe the main achievements of medical science as seen against a historical background. Their 17 expert contributors have served them well in identifying the important advances made in the control of infections, in anaesthesia, and in surgery and many new techniques of

seem to be singularly one-sided affairs. The reason may be that the arthropods are, for the most part, recent additions to the fauna of the human skin. The germs they carry may be better adapted to mammals other than man. Both vectors and micro-organisms may not yet have had time to develop anything like a symbiotic relationship with man.

There is in fact some suggestion that such relationships may be evolving. Although the arthropod parasites may not benefit man they do seem, in the majority of cases, after an initial rather heavy infestation, to decrease in numbers to produce a balanced, rather small population which does not cause intolerable discomfort. Thus the majority of lousy children, including those with long-term chronic infestations, have only about a dozen lice on their heads. Most patients with chronic scabies support only a similar number of *Sarcoptes* mites. Perhaps the idea, prevalent among Victorian slum dwellers, that a few lice on a child's head was a sign of health may have some foundation. Perhaps low and relatively harmless populations of ectoparasites prevent other and more dangerous invaders from damaging our skin. The ecological ideas put forward by Michael Andrews in this excellent book may stimulate scientists to investigate these problems anew.

Kenneth Mellanby

Although Professor Mellanby was at one time a Reader in Medical Entomology, he has become better known for his work at the Monks Wood Experimental Station, Huntingdon, UK, and for his success in conveying the complexities of science to a wider audience.

diagnosis and treatment. They have also explained the growth in our understanding of the structure and biochemistry of the body in health and disease. Although some chapters, such as Professor Roy Calne's account of the kidney and of organ transplantation are models of clarity, others—though technically competent—are fogged by patches of the impenetrable jargon characteristic of so much medical writing. Perhaps because the editors realised that too many of the contributions were too detailed for a non-specialist readership they included a glossary of technical terms; but even that assumes a considerable scientific vocabulary.

Books of this kind stand or fall on their appeal to the eye. A high standard has been set by designers such as George Rainbird (responsible for successes such as Douglas Botting's biography *Humboldt and the Cosmos*), whose formula depends on the faultless reproduction of large numbers of plates of exceptional beauty, many of

Photo: Mansell Collection

them in colour. In contrast, the reader browsing through *Triumphs of Medicine* finds no colour; and though every page has a picture and many are full plates, few are really eye-catching. Many of the full-page illustrations are taken from X-ray films; again, their technical quality is excellent, but few non-medical readers will find them informative. Virtuoso examples of the radiologist's skills, such as a film showing exactly where blood is leaking from an artery into the intestine, mean little to someone who has never seen an angiogram before. The same criticism may be made of the photomicrographs; without explanation they are meaningless to the non-medical eye and they have little visual impact.

There is an exciting story to be told in the growth of medical expertise as part of the twentieth century explosion of scientific knowledge, but the telling must be adapted to the audience. Here the target is far from clear, for a collection of intellectually demanding essays has been confused and interrupted by a haphazard mixture of technical illustrations, historic cartoons, line drawings, portraits, and journalistic photographs. The packaging is not consistent with the content, and in consequence the result will please neither the specialist reader nor the curious layman. **Tony Smith**

Tony Smith is Assistant Editor of the British Medical Journal and Medical Correspondent of The Times.

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Islamic masterwork

Islam and the Arab World. Edited by Bernard Lewis. Pp. 360. (Knopf in association with American Heritage: New York, 1976.) \$35.00. (UK edition (*The World of Islam*) published by Thames and Hudson: London, 1976. £12.50.)

THE publication of this first American edition of *Islam and the Arab World* is truly an occasion for celebration.

Furthermore, the celebrants need not be Arabic or Islamic scholars alone, for the volume is sure to be appreciated by the complete spectrum of professionals, from artists to zoologists. At a casual glance, this profusely and beautifully illustrated book might appear to be a classier version of a typical multi-colour 'coffee-table' book, which are more often valued for their role as icebreakers in a conversation, rather than for their intrinsic value. That would indeed be an incorrect impression because in the (effectively) fifteen chapters of the book, the reader is gently but carefully guided through topics such as the explosion of Islam in the Arabian peninsula and beyond, the art and architecture of the vast Dār al-Islām, ranging from Andalusia to Bengal, its literature, natural sciences, music and a bird's-eye view of its up-to-date political history. Each chapter is written by unquestionable authorities on their subjects, seemingly handpicked by Professor Bernard Lewis not only for their scholarship but also for their ability to communicate with the lay public and, what is more important, for their unusual sense of balance. In fact, there are perhaps only one or two chapters which occasionally show lapses in this latter mentioned quality.

The title of the book could be slightly misleading in that a potential reader may not realise the existence of whole chapters in the book dealing with Muslim Spain, Iran from the Safavids onwards, the Ottoman empire, and last but not least, Muslim India. Thus, in the strict definition of the Arabist, the present volume deals with the whole of Islamic culture (or "Islamicate" culture as the late Marshall Hodgson would have said) and not just Arabic civilisation. The corollary of that is the book's involvement with the whole of Dār al-Islām since the Prophet's birth.

The chapter entitled "The Scientific Enterprise" deserves special notice because of the sparkling characteristics of this long essay. It is well-known to students of Islamic science that other than the articles in *Encyclopedia of Islam* (incomplete second edition) or the *Legacy of Islam* (1974), there is not even a satisfactory monograph on the subject of Islamic science. Obviously, Professor Sabra's article in twelve pages (even if they are quarto-size pages) does not remove this inadequacy, but it goes a long way towards initiating an intelligent and non-partisan discussion about the paradox of the coexistence of rationalists (falasifa) and the orthodox religious thinkers (Ahl as-Sunnah).

Although the book is a masterwork, I would like to slip in a few minor criticisms here: the dust cover is too garish and projects too narrowly martial an image for such a world culture; the excellent chapter on music does not mention a word about Hindustani music; and for a confirmed epicure it is distressing to find not only a lack of mention of Turkish cuisine which vies with the French and Chinese as a primary world cuisine but that even the word 'food' is never mentioned. Perhaps the coffee-table culturists would at least have liked to know the origin of the word 'coffee'!

Subir K. Banerjee

Subir Banerjee is Professor in the Department of Geology and Geophysics at the University of Minnesota, Minneapolis, Minnesota, and has recently been appointed an Adjunct Professor in the Program of Middle Eastern and Islamic Studies at that University.

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